

cordance with the feeble hydrolytic dissociation of the organic salt (*infra*) Citrate solutions are known to have but little buffer value (a fact not taken into account by Mellon, 1922) and some simple tests were set up to illustrate this point

B Two test tubes, each containing 10 cc of the usual phosphate buffer mixture at $pH = 7.2 + 5$ drops of 0.2 per cent phenol red indicator, were placed in Cole Omslow comparator along with another similar tube containing a like amount of phosphate and indicator at $pH, 7.4$ A four month old 10 per cent solution of sodium citrate was run into one of the $pH, 7.2$ tubes and a corresponding amount of distilled water into each of the other tubes After the addition of 6 or 7 cc of the citrate solution a color change became detectable (as compared with the control) Some 14 or 15 cc of citrate was required before the color matched that in the (watered) $pH, 7.4$ tube

C A specimen of rabbit venous blood (collected under oil) was subdivided between tonometers containing, respectively, (a) one half, (b) twice, and (c) five times as much of (the same) 10 per cent sodium citrate as of blood The corresponding pH values, with the glass electrode (no gas leakage), were (a) 6.91, (b) 7.01, and (c) 7.03 Although time intervals were not recorded in this experiment, which, therefore, takes no cognizance of the "acid shift" noted above, nevertheless, the determinations were all made within an hour or two and, in all probability, do indicate the alkalinizing effects of large amounts of citrate solution

DISCUSSION

I *pH During Blood Coagulation*—Hober (1903) could find no pH change during blood clotting He used the type of hydrogen electrode with which Michaelis (1914) recorded an acid shift due to "the deposition of fibrin on the platinum" Ross (1921), using the dialysis technic of Dale and Evans (1920) which employed an indicator method said to be "of the same order of accuracy as the H electrode," found no change (within $+0.02$ pH) in oxalated blood before and after recalcification Kugelmass (1922), using a H electrode reported an "alkaline shift" of $pH = 0.3$ during the clotting of diluted oxalated plasma and prepared fibrinogen by a purified thrombin solution He made no effort to control (*inter alia*) the gas equilibria Hirsch (1924) used a delicate colorimetric method of determining pH by means of an indicator (cresol red) and the Keuffel Esser color analyzer Representative color readings were 51 per cent at $pH = 7.45$ and 60 per cent at $pH = 7.36$ The quoted pH values represent the average determinations on plasma and serum (respectively) in 10 samples of rabbit heart blood collected, centrifuged, and examined under a layer of paraffin oil to prevent gas loss The acid change, averaging 0.09 pH , was not observed prior to clotting in 3 cases which failed to coagulate in half an hour, but it was detected before that time in several samples which clotted early Havard and Kerridge (1929), using the glass electrode (Kerridge's pattern, 1926) to investigate acid changes in shed blood (see later) noted, incidentally, that clotting in the electrode pipette made no difference to the pH time curves obtained In some experiments at room temperature the pH remained constant during coagulation Laug, 1934 (*infra*), with the Stadie

(1931) glass electrode, also recorded that clotting in the apparatus failed to modify these "acid-shifts." Eagle and Baumberger (1935) used the glass electrode of Baumberger to demonstrate the absence of pH change in the clotting of fibrinogen by thrombin.

Our experience with the DuBois glass electrode follows up the lead of these recent workers and helps to establish beyond a reasonable doubt that no significant change in pH (with instruments able to register accurately to within 0.005 or 0.01 of a pH unit) results from blood coagulation. Dismissing Kugelmass' anomalous and poorly controlled data, it would seem reasonable to explain Hirsch's results on the basis of the "acid-shift" which we shall review in the succeeding section.

II. *The "Acid-Shift" in Shed Blood.*—Lovatt Evans (1922) reviewed the earlier literature, dating from Zuntz (1868), to show that the CO₂ capacity of shed blood was highest immediately after withdrawal and usually suffered considerable reduction when the blood was allowed to stand outside the body. He gave good evidence that the chief cause of the phenomenon was the accumulation of lactic acid due to glycolysis. The "acid-shift" was irregular in incidence and magnitude. It did not occur in separated plasma but only in the presence of corpuscles, especially the leucocytes (Maclean and Weir, 1915). It was accelerated by previous lowering of the CO₂-tension (Mellanby and Thomas, 1920; Henderson and Haggard, 1920). Evans found that temperature played an important rôle. An initial chilling of the blood greatly retarded glycolysis and the acid change. Even without cooling it could be checked for at least half an hour by 0.4 per cent of oxalate (Macleod, 1913) plus 0.1 per cent of fluoride. A small correction (0.9 vols. per cent for each 0.1 per cent NaF) was necessitated by the reduction in CO₂ capacity by the added fluoride itself.

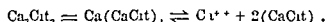
Havard and Kerridge (1929), without disturbing the gas equilibria, measured the pH of whole blood (at 38° C.) using Kerridge's (1926) modification of the bulb-type glass electrode. They, too, observed an acid-shift averaging pH = 0.05 which set in within two or six minutes (sometimes longer) after the blood had been drawn and held in the electrode. Clotting did not alter the pH:time curves. Blood corpuscles were necessary, the serum and plasma showing no pH variations, although centrifuged laked blood did. The acid change was markedly retarded at room temperature and completely stopped at 0° C. It was not affected by substances which inhibited (a) coagulation (oxalate, fluoride, heparin), and (rather anomalously) (b) glycolysis (NaF, 0.06 per cent). After a flattening out of the pH:time curves a later acid drift developed, leading them to emphasize the earlier alterations as a "first acid change." A few indefinite attempts at lactic acid estimations (and the foregoing considerations) caused them to avoid any correlation of the acid change with Evans' electrolytic reaction.

Lang recently reinvestigated the problem with the aid of Stadie's (1931) glass electrode and careful controls. He could not separate two phases of acid production but found that the change occurred from the commencement of observation (two and one-half minutes after withdrawal of blood) and amounted to an average (41 samples of whole blood at 38° C.) of 0.008, 0.007, 0.004,

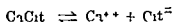
0.004 (of a pH unit) in successive five minute intervals. The data pointed to a continuous "acid shift" running parallel with lactic acid production. The fact that the change was most marked in the first ten minutes suggested that glycolysis was especially vigorous at the outset. Fluorides were able to check the acid change if present in sufficient concentration but 1 or 2 per cent might be required instead of the 0.05 or 0.1 per cent concentrations usually employed. He again confirmed the importance of temperature, but gave good arguments against the possibility that the acid shift was a mere "temperature artefact" as suggested by Platt and Dickinson (1933). Laug also adduces evidence which, on the whole, negates the possibility that electrical charges due to red blood cell sedimentation ("sedimentation artefact") play any significant rôle in the acidity phenomenon, although a definite drift, attributed to this cause, complicated some of his readings.

The DuBois pipette electrode in its present form is a modification of the MacInnes glass electrode. It has an active diameter of less than 0.5 mm. and a very low resistance, averaging below 5 megohms. For blood measurements the outside of the electrode tip is ground to fit snugly into the top of the capillary tube containing the blood. The blood chamber is kept at 38° C. by a warming jacket permitting a flow of water at the desired temperature, and temperature fluctuations of the blood sample, when transferred immediately, as in the present experiments, are minimal. The plugging of the blood tube by the pipette prevents the escape of CO₂, and, since the glass membrane is at the top of the blood column, its potential is unaffected by the drift or settling of the red blood cells. We feel, therefore, that the artefacts discussed by Laug may be ruled out in our experiments. The present data agree with those of Laug and of Havard and Kerridge in identifying a true "acid shift" in shed blood. In the light of the literature cited, albeit the possibilities of oxidative phenomena and bacterial action have not received serious consideration, we may interpret the acid change in terms of a glycolytic phenomenon.

Our additional finding of a slight acidifying effect of small quantities of trisodium citrate bears out the recent work of Hastings, McLean, Eichelberger, Hall and DaCosta (1934) on the ionization of calcium citrate. Estimating calcium ion concentration by the new frog heart technique, they demonstrated clearly that calcium citrate ionizes in two stages. The primary dissociation is almost complete and follows the equation



The secondary dissociation, being that of a weak electrolyte, is incomplete, and according to the equation



At 22° C., pH = 7.4, and total ionic strength of $\mu 0.155 - \mu 0.163$, the electrolytic dissociation constant, $\text{p}K_{\text{CaCit}}$ was computed to be 3.22 ± 0.025 . The scanty data in the literature on calcium citrate conductivity were quoted by these authors in support of the idea that such complex citrate ions do exist.

Explicable also on the basis of the foregoing data is the observation of Greenberg and Greenberg (1932) that citrates caused calcium to migrate to the anode in electrodialysis of serum ultrafiltrates.

The alkalinizing effects of *large* quantities of citrate probably depend upon interionic equilibria such as are invoked to explain loss of calcium by precipitation during plasma determinations (Cameron and Moorhouse, 1925).

SUMMARY

1. pH determinations with the sensitive glass electrode demonstrate no change during blood coagulation.

2. The pH of blood samples preserved in mercury tonometers at room temperature, with no attention to bacteriologic sterility, glycolysis, or oxidative phenomena, shows a pronounced "acid-shift" during a twenty-four-hour observation period. There are indications that the change may commence within half an hour. Data in the literature point to lactic acid production by glycolysis as the major cause of the acid change.

3. Trisodium citrate in small quantities (used as an anticoagulant) consistently gave samples slightly more acid than untreated (clotted) blood preserved for corresponding periods. This is explained on the basis of a primary dissociation into CaCit^- anions.

4. The "acid-shifts," due to both time and citration, persisted in samples subsequently alkalinized by aeration.

5. H-electrode determinations of the pH of various citrate solutions gave values ranging between 7.5 and 8.5. An old 10 per cent solution was sufficiently alkaline for proportions (citrate:diluent) in excess of 1:2 to give an "alkaline shift" to (a) phosphate buffer mixture and (b) rabbit venous blood.

Besides settling a controversial question concerning blood coagulation, these data have several practical applications, first, to the preservation of blood samples for pH determination, and second, in connection with the problems of human blood transfusion by the citrate method.

REFERENCES

1. Cameron, A. T., and Moorhouse, V. H. K.: The Tetany of Parathyroid Deficiency and the Calcium of the Blood and Cerebrospinal Fluid, *J. Biol. Chem.* 63: 687, 1925.
2. Du Bois, D.: A Vacuum Tube Potentiometer Applicable for Use With Glass Electrodes of High Resistance, *J. Biol. Chem.* 89: 729, 1930.
3. Du Bois, D.: A Glass Electrode for Testing the pH of Blood, *Science* 76: 441, 1932.
4. Dale, H. H., and Evans, C. L.: Colorimetric Determination of the Reaction of Blood by Dialysis, *J. Physiol.* 54: 167, 1920.
5. Eagle, H., and Baumberger, J. P.: Studies on Blood Coagulation. IV. On the Constancy of the Hydrogen Ion Concentration During the Coagulation of Fibrinogen by Thrombin, *J. Gen. Physiol.* 18: 809, 1935.
6. Evans, C. L.: Acid Production in Shed Blood, *J. Physiol.* 56: 146, 1922.
7. Hastings, A. B., McLean, F. C., Eichelberger, L., Hall, J. L., and Da Costa, E.: The Ionization of Calcium, Magnesium, and Strontium Citrates, *J. Biol. Chem.* 107: 351, 1934.
8. Havard, R. E., and Kerridge, P. T.: Immediate Acid Change in Shed Blood, *Biochem. J.* 23: 600, 1929.
9. Henderson, Y., and Haggard, W. D.: Haemato-Respiratory Functions. IX. An Irreversible Alteration of the $\text{H}_2\text{CO}_3:\text{NaHCO}_3$ Equilibrium of Blood, Induced by Temporary Exposure to a Low Tension of CO_2 , *J. Biol. Chem.* 45: 209, 1920.
10. Hirsch, E. F.: Changes in Hydrogen Ion Concentration of Blood With Coagulation, *J. Biol. Chem.* 61: 795, 1924.
11. Hober, R.: Ueber die Hydroxylionen des Blutes, *Pflügers Arch.* 87: 522, 1900.
12. Idem: 99: 572, 1903.
13. Kerridge, P. T.: (a) The Use of the Glass Electrode in Biochemistry, *Biochem. J.* 19: 611, 1925; also (b) *J. Sc. Inst.* 3: 404, 1926.
14. Laug, E. P.: Reinvestigation of Phenomenon of First Acid Change in Whole Blood, *J. Biol. Chem.* 106: 161, 1934.

- 15 Maclean, H, and Weir, H B The Part Played by the Different Blood Elements in Glycolysis, *Biochem J* 9 412 1915
- 16 Macleod, J I R Blood Glycolysis Its Extent and Significance in Carbohydrate Metabolism The Supposed Existence of "su re virtuel" in Freshly Drawn Blood, *J Biol Chem* 15 497, 1913
- 17 Mellanby, J, and Thomas, C I Carbon Dioxide Carrying Power of Constituents of Plasma Alkali Reserve of Blood *J Physiol* 54 178, 1920
- 18 Mellon, R R, and Co Workers *J A M A* 78 1024 and 1026, 1922, *Ibid* 79 1678 1922
(Williams, J R, and Sweet M Hydrogen Ion Concentration Studies on Distilled Water, Physiologic Sodium Chlorid, Glucose, and Other Solutions Used for Intravenous Medication)
- 19 Mellon, R R, Slagle, E A, and Aere S F The Practical Application of "Buffers" in the Regulation of the Hydrogen Ion Concentration of Intravenous Solutions
- 20 Mellon R R, Hastings, W S, and Carey, G U Observations on the Effect of Sodium Citrate on the Blood)
- 21 Michaels L, and Rona, P Elektrochemische Alkalinitäts Messungen in Blut und Serum, *Biochem Ztschr* 18 317 1909
- 22 Michaels, L Die Wasserstoffionen Konzentration I 145, Berlin 1914
- 23 Platt, B S, and Dickenson S The Technique of Glass Electrode Measurements, *Biochem J* 27 1068 1933
- 24 Ross, J P The Influence of Coagulation Upon the Reaction of the Blood, *J Physiol* 54 lxxxviii (*Proc Physiol Soc*) 1921
- 25 Stadie W C An Electron Tube Potentiometer for the Determination of pH With the Glass Electrode, *J Biol Chem* 83 477, 1929
- 26 Stadie W C O'Brien H and Irig E P Determination of the pH of Serum at 38° With the Glass Electrode and an Improved Electron Tube Potentiometer, *J Biol Chem* 91 243, 1931
- 27 Zuntz, N *Beitr z Physiol d Blutes*, p 25, Bonn, 1868

AURICULAR FLUTTER WITH 1:1 RESPONSE*

A CASE REPORT

J. BAILEY CARTER, M.D., AND EUGENE F. TRAUT, M.D., CHICAGO, ILL.

AURICULAR flutter, although more rarely encountered, is closely related to auricular fibrillation. Flutter is believed¹ to result from "circus movement," i.e., a wave of contraction circulating regularly and continuously through the auricle. Usually, if untreated, it continues unchanged for months or years.⁶ Instances are known¹ in which the auricles beat without cessation at a regular rate of 280 per minute for a period of ten or more years, a 2:1 block causing a ventricular rate of half the above, the patient meanwhile showing little change. Rarely it occurs in short paroxysms.¹ In Table I, "established flutter" indicates failure of the arrhythmia to end spontaneously within fourteen days,⁶ "paroxysmal flutter" denotes briefer attacks.⁵ Symptoms of this persistent condition resemble those of auricular fibrillation. The prognosis in flutter is much the same as in fibrillation. The reaction of the heart depends on its reserve and on the nature of the burden imposed by the flutter.¹ It is important to remember that flutter is very amenable to proper treatment.

The auricular rate in flutter is usually 260 to 320 per minute.¹ The ventricles commonly respond to each alternate auricular beat: the ventricular rate being half the auricular rate or 130 to 160 per minute. A certain degree of block is usually associated. This varies. There may be complete auriculo-ventricular dissociation,^{2, 3} 4:1, 3:1, 2:1 block, while infrequently each auricular impulse provokes a contraction of the ventricles. The ventricular rate becomes the same as that of the rapidly beating auricles.

A patient with 2:1 flutter, although conscious of palpitation, is often able to lead practically a normal life. He may, however, suddenly become a victim of these paralyzing attacks of 1:1 flutter. Such 1:1 response occurs whenever the refractory period of the A-V node becomes less than the length of one auricular cycle.⁵ These paroxysms at first may occur only once a week. Gradually they become more frequent, requiring less and less to precipitate them. Finally they occur daily or oftener, even with the patient at rest in bed.¹⁸ In 1:1 flutter, the junctional tissues have been known to transmit stimuli up to a rate of 320 per minute,⁴ the fastest human ventricular rate ever recorded. It is a higher rate of frequency than is recorded in any other type of cardiac arrhythmia.⁵

In a critical and exhaustive survey of the literature, Bedell⁵ reports 16 instances of authentic auricular flutter with 1:1 response. To these she adds two additional cases. This is preponderantly a series of middle-aged pa-

*From the Medical Service of Cook County Hospital and the Department of Cardiology, Rush Medical College of the University of Chicago.

Received for publication, May 20, 1935.

tients⁵ Sixteen patients were males and three were females, bearing out the statement of Lewis⁷ that flutter is four times as common in males as in females. The frequency of cases with 1 1 attacks among all patients with auricular flutter is reported to be 4 per cent^{5, 6}. The etiologic factors are the same as those responsible for flutter in general—rheumatism, arteriosclerosis, syphilis, hypertension, hyperthyroidism, or any other disease resulting from toxin, infection, or otherwise. A 2 1 block predominated in all of these patients with 1 1 paroxysms.

The attacks usually follow some form of exertion or excitement. Paroxysms have followed the administration of quinidine⁶ or a combination of morphine, atropine, and avertin⁵. The onset is sudden with an immediate jump from 2 1 to 1 1 response. Extreme weakness, palpitation and shortness of breath are the usual manifestations. These symptoms force the ambulatory patient to sit down or may cause the patient in bed to sit up in order to breathe. The palpitation is usually overshadowed by the other symptoms and may be overlooked by the patient in giving the history of an attack. Precordial pain may be present as in Cases 3, 11, and 16. It may radiate to the epigastrium or down the left arm. Tremor, sweating, vertigo, nausea and great mental agitation are frequently felt. Usually syncope does not occur but consciousness may be lost, as in Cases 7, 12, and 15. Congestive failure is not common but may occur as in Case 7. The patient is usually unable to stir during the attack, Case 4 being a remarkable exception. Paroxysms are usually transitory. In the majority of instances their duration is from thirty minutes to two hours. An attack may last only a few minutes or a number of hours. The offset of an attack is usually gradual. The two grades of block intermingle. A gradual transition from one heart rate to the other is thereby provided. Most attacks end spontaneously. At times therapy has been effective as in Cases 5, 7, 15, and 19. Bedell⁵ reports the only instance of 1 1 flutter, established by electrocardiograms, in which a complete autopsy has been performed. The attacks of syncope and the possibility of congestive failure during frequent paroxysms somewhat modify the general prognosis in flutter⁵. The most important remedy in flutter is digitalis or an allied drug¹. Digitalis effectively prevents 1 1 paroxysms by increasing the A V block. "Quinidine employed in flutter enjoys no great success."¹ It has been held responsible for paroxysms of 1 1 response in flutter^{5, 6}. Flutter was effectively controlled by quinidine in the case here reported.

CASE HISTORY

White, male, aged sixty years, was admitted to Cook County Hospital, May 17, 1933. He complained of attacks of palpitation following influenza one year previously. The attacks became more frequent and had confined him to bed for several months because of their daily recurrence. These paroxysms usually followed exertion—walking, straining at stool, or sitting upright in bed. The attacks were of sudden onset, the heart continuing to pound until he would lie down, after which there would be a couple of very "large thumps" and then the attack would subside altogether. The patient himself discovered that flexing the head and putting his hand against the carotid artery stopped the attacks. Vertigo was noticed during these attacks. There were no other complaints. He had been confined to bed for several weeks with rheumatism at the age of eleven years. He admitted having had gonorrhea at the age of thirty years. Syphilis was denied.

TABLE I
SUMMARY OF CASES OF ATRIAL FLUTTER WITH 1:1 RESPONSE. MODIFIED FROM IDELL'S CHART

NO.	REF.	AUTHOR AND DATE	SEX	COL- OR	AGE	CARDIAC DIS- EASE	ASSOCIATED DISEASES	TYPE OF FLUT- TER	A V RATIOS	DURA- TION	1:1 RATE	REMARKS
1	5	Mackenzie, 1910-1911	M	W	47	None	Mild typhoid before onset	Estab. and Parox.	1:1, 2:1, 3:1	11 yr.	290 300	Attacks following exertion and defor- mation; controlled by NH ₄ Br. Dig- italis restored normal sinus rhythm
2	9	Koplik, 1917	M	W	10	None	None	Estab.	1:1, 2:1, 3:1, 3:2	2 mo.	225	Marked congestive failure during 1:1 attacks
3	1	Blackford and Willius, 1918	M	W	15	Heart enlarged	Typhoid when 18 yr. old	Estab.	1:1, 2:1	2 yr.	224	Attacks of dyspnea-precordial pain ra- diating to epigastrium. Incompensat- ing. Duration ½ hr.
4	1	Blackford and Willius, 1918	F	W	32	None	Tonsillitis-ex- oph. goiter. Biliary colic	Estab.	1:1, 2:1	Several months	320	During one attack ventricular rate was 320 for nearly 2 hr. Patient walked several blocks
5	10	Scott, 1922	M	W	25	None	Influenza pneu- monia 2 yr. before	Estab.	1:1, 2:1	Few months	272	Attacks on slight exertion. Ended by vagal pressure—lying flat. Digitalis induced sinus rhythm. No flutter for 1½ yr.
6	11	Wold, 1924	M	W	47	Tympanic aortic second	None	Estab.	1:1, 2:1	6 wk. 117 yr.	260	Attacks on exertion or excitement. Sim- ilar attacks for one month 17 years before
7	12	Gallavardin, 1924	M	W	47	None	Fibroid pulm. tuberculosis	Estab.	1:1, 2:1, 3:2	5 mo. 330 yr.	270	Attacks following any effort. Once fell on the street
8	13	MacMillan, 1925	M	W	57	Aneurysm and aortitis	Syphilis	Estab.	1:1, 2:1	3 wk. plus	260	Attacks on mild exertion. Duration of attacks, 3 to 5 min.
9	11	Harard, 1926	F	W	??	Compensated mitr. sten. aort. insuf.	Rheumatism denied	Estab.	1:1, 2:1, 3:1, 4:1	6 mo. 42-3 yr.	250	Attacks on emotion or slight exercise. Digitalis produced 5:1-7:1 block. No more attacks
10	15	Poynton, 1926	M	W	31	Heart enlarged	None	Parox.	1:1, 2:1, 3:1	31 yr.	250	Frequent attacks since birth. Attacks lasted several days

TABLE I—CONT'D

NO	REF	AUTHOR AND DATE	SEX	COI OF	AGE	CARDIAC DIS EASE	ASSOCIATED DIS EASES	TYPE OF FLUTTER	NO. OF ATTACKS	DURA TION	1:1 RATE	REMARKS
11	6	Parkinson and Bedford, 1927	M	W	43	Mitral stenosis Aortic insuff	None	I stab	1:1, no others stated	5 mo	254	Attacks for 20 min to 2 hr. Pain about left nipple and down left arm
12	6	Ibad, 1927	M	W	55	Aortic scler	Syphilis	?	1:1, 2	Unknown	275	Two attacks with syncope
13	16	Herspath, 1928	M	W	28	?	None	Estab	1:1, 2:1	37 yr	261	Invalided out of army After 5 yr well. Then 2 attacks of flutter in next 2 yr
14	16	Herspath, 1928	M	W	17	None	Followed in influenza	Estab	1:1, 2:1	8 mo	288	Attacks produced by exercise. No EKG of arrhythmia
15	17	Sprague and White, 1928	M	W	49	Heart slightly enlarged	Migraine	I stab	1:1, 2:1	5 yr	210	Attacks on exertion—emotion, min to hr, sometimes stopped by holding full inspiration. Once fell to floor at onset
16	18	Fuller, 1928	M	W	16	Hypertension	None	Estab	1:1, 2:1, 3:1	1 yr	210	Weak spells on exertion or excitement—later during rest
17	5	Bedell, 1933	F	C	40	Heart enlarged	Exophthalmic goiter	Estab	1:1, 2:1, 3:1	8 mo	260	Patient foramen ovale found it on top
18	5	Bedell, 1933	M	C	55	Renal disease prenatally	None	Estab	1:1, 2:1, 3:1	6 mo	222	Possible attack 10 yr before. Attacks on exertion
19	00	Carter and Traut, 1934	M	W	60	Followed influenza	None	Estab	1:1, 2:1, 3:1	1 yr	200	Vagus effect stopped attacks. Quinine controlled flutter

TABLE I
SUMMARY OF CASES OF AURICULAR FLUTTER WITH 1:1 RESPONSE, MODIFIED FROM REDDILL'S CHART

NO.	REF.	AUTHOR AND DATE	SEX	COLOR	AGE	CARDIAC DISEASE	ASSOCIATED DISEASES	TYPE OF FLUTTER	A-V RATIOS	DURATION	1:1 RATE	REMARKS
1	8	Mackenzie, 1910-1911	M	W	47	None	Mild typhoid before onset	Estab. and Parox.	1:1, 2:1, 3:1	11 yr.	290 to 300	Attacks following exertion and defecation; controlled by NH ₄ Br. Digitalis restored normal sinus rhythm
2	9	Koplik, 1917	M	W	10	None	None	Estab. ?	1:1, 2:1, 3:1, 3:2	2 mo.	225	Marked congestive failure during 1:1 attacks
3	4	Blackford and Willis, 1919	M	W	45	Heart enlarged	Typhoid when 18 yr. old	Estab. ?	1:1, 2:1	2 yr.	224	Attacks of dyspnea-precordial pain radiating to epigastrium. Incapacitating. Duration ½ hr.
4	4	Blackford and Willis, 1919	F	W	32	None	Tonsillitis-exoph. goiter. Biliary colic	Estab.	1:1, 2:1	Several months	320	During one attack ventricular rate was 320 for nearly 2 hr. Patient walked several blocks
5	10	Scott, 1922	M	W	25	None	Influenzal pneumonia 2 yr. before	Estab.	1:1, 2:1	Few months	272	Attacks on slight exertion. Ended by vagal pressure—lying flat. Digitalis induced sinus rhythm. No flutter for 1½ yr.
6	11	Wedd, 1924	M	W	47	Tympanic aortic second	None	Estab.	1:1, 2:1	6 wk. 717 yr.	260	Attacks on exertion or excitement. Similar attacks for one month 17 years before
7	12	Gallavardin, 1924	M	W	47	None	Fibroid pulm. tuberculosis	Estab.	1:1, 2:1, 3:2	5 mo. 230 yr.	270	Attacks following any effort. Once fell on the street
8	13	MacMillan, 1925	M	W	57	Aneurysm and aortitis	Syphilis	Estab.	1:1, 2:1	3 wk. plus	260	Attacks on mild exertion. Duration of attacks, 3 to 5 min.
9	14	Bernard, 1926	F	W	??	Compensated mitr. sten. aort. insuf.	Rheumatism denied	Estab.	1:1, 2:1, 3:1, 4:1	6 mo. 22-3 yr.	250	Attacks on emotion or slight exercise. Digitalis produced 5:1-7:1 block. No more attacks
10	15	Poynton, 1926	M	W	34	Heart enlarged	None	Parox.	1:1, 2:1, 3:1	3½ yr.	250	Frequent attacks since birth. Attacks lasted several days

TABLE I—CONT'D

NO	REF	AUTHOR AND DATE	SEX	COI OP	AGE	CARDIAC DIS EASI	ASSOCIATED DIS EASES	TYPE OF FLUTTER	AV PATIOS	DUR A TION	1:1 RATIO	REMARKS
11	6	Parkinson and Bedford, 1927	M	W	43	Mild sten Aort insuff	None	Fstrib	1:1, no others stated	5 mo	254	Attacks for 20 min to 2 hr Pain about left nipple and down left arm
12	6	Isard, 1927	M	W	55	Aort scler	Syphilis	1	1:1, '	Un known	275	Two attacks with syncope
13	16	Herrapath, 1928	M	W	28	?	None	Estrib	1:1, 2:1	77 yr	264	Invalided out of army After 5 yr well Then 2 attacks of flutter in next 2 yr
14	16	Herrapath, 1928	M	W	17	None	Followed in fluenza	Fstrib	1:1, 2:1	8 mo	288	Attacks produced by exercise No EKG of arrhythmia
15	17	Sprague and White, 1928	M	W	49	Heart slightly enlarged	Migraine	Estrib	1:1, 2:1	5 yr	240	Attacks on exertion—emotion, min to hr, sometimes stopped by holding full inspiration Once fell to floor at onset
16	18	Fuller 1928	M	W	46	Hypertension	None	Fstrib	1:1, 2:1, 3:1	1 yr	240	Weak spells on exertion or excitement —later during rest
17	5	Bedell, 1933	F	C	40	Heart enlarged	Exophthalmic goiter	Fstrib	1:1, 2:1, 3:1	8 mo	260	Patent foramen ovale found at autopsy
18	5	Bedell, 1933	M	C	55	Rare auric pre mit beat	None	Dstrib	1:1, 2:1, 3:1	6 mo	222	Possible attack 10 yr before Attacks on exertion
19	00	Carter and Traut, 1934	M	W	60	Followed in fluenza	None	Dstrib	1:1, 2:1, 3:1	1 yr	200	Vagus effect stopped attacks Quinine controlled flutter

He was well nourished. There was slight cyanosis of the lips and finger nails. Pupils reacted promptly to light and in accommodation. Few remaining teeth in fair condition. Tonsils moderately enlarged. No cervical lymphadenopathy. Thyroid normal. Cervical veins not engorged. Heart slightly enlarged. Lower sternum resonant. Radial pulse slow and irregular. Apical pulse regular, except for an occasional dropped beat. No murmurs or

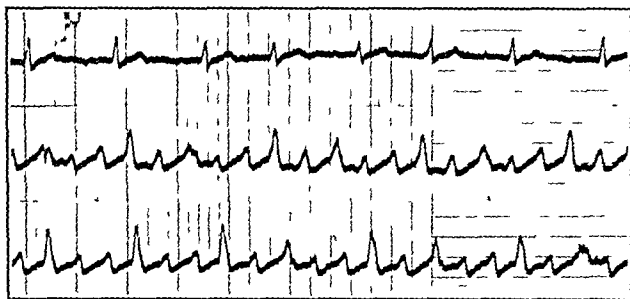


Fig. 1.—Recorded 5/20/33. Before exercise. Auricular flutter with 2:1 and 3:1 ventricular response. Auricular rate 210 per minute. Ventricular rate 80 per minute.

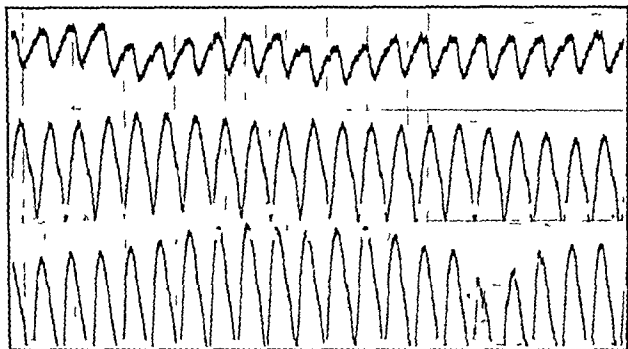


Fig. 2.—Recorded 5/20/33. After rising in bed ten times. Auricular flutter with 1:1 ventricular response. Rate 210 per minute.

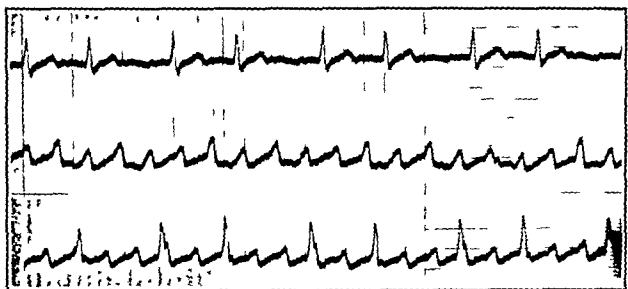


Fig. 3.—Recorded 5/23/33. Before exercise. Similar to Fig. 1

abnormal accentuations. Blood pressure 128/94. Chest resonant; anteroposterior diameter increased. Lungs clear. Liver and spleen not palpated. No edema. Reflexes normal.

Urine examination: specific gravity of 1.020, alkaline in reaction, and an occasional granular cast. Blood examination, Hb 95 per cent, R.B.C. 5,660,000, and W.B.C. 6,150. Blood Wassermann and Kahn tests were negative. X-ray of chest revealed slight cardiac enlargement.

Progress.—May 18, 1933: Diagnosis was left ventricular hypertrophy due to ancient rheumatism with paroxysmal tachycardia or fibrillation. May 19, patient had attack while straining at stool and again while walking. Rate was 200 per minute and regular. First attack was controlled by continued pressure on supraorbital nerve; the rate changed to 100 and irregular. The second time supraorbital pressure failed to decrease the rate but sitting

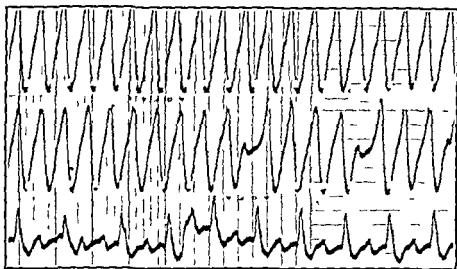


Fig. 4.—Recorded 5/23/33. After exercise. Effect of supraorbital pressure apparent in Lead 2. Cessation of the attack of 1:1 response followed. Flutter with 2:1 response recorded in Lead 3.

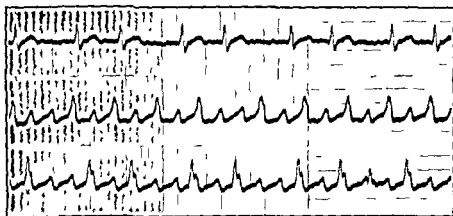


Fig. 5.—Recorded 5/29/33. Before intensive quinidine medication.

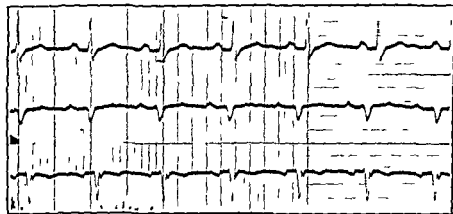


Fig. 6.—Recorded 6/4/33. After intensive quinidine medication

down relieved the attack. May 20, pulse irregular and slow with alternate strong and weak beats. May 24, frequent attacks of tachycardia. Alternate beats dropped at radial pulse. May 25, attacks more frequent today. Quinidine sulphate gr. *ni* t.i.d. May 26, no attacks today. May 27, diagnosis of auricular flutter. Quinidine increased to gr. *v* q.i.d. May 28, definitely improved. Heart regular at a rate of 90 per minute. No attacks since yesterday. May 29, questionable attack yesterday. May 30, no attacks for two days. Pulse

slow and regular. May 31, feels fine. No paroxysms of tachycardia. Still on quinidine gr. v q. i. d. June 1, paroxysm while straining at stool today. Otherwise pulse 60, with coupling of beats at times. June 2, paroxysm on walking. June 3, quinidine gr. v, every hour for 12 doses. June 4, pulse slow and regular. June 6, no paroxysm since intensive quinidine medication, even on walking about the ward. Discharged. To return to cardiac clinic. June 8, walked four miles to cardiac clinic in one and three-quarters hours. Feels fine. Pulse regular. Unable to contact patient since.

COMMENT

Our patient had an established auricular flutter with a suggestive history and characteristic electrocardiographic findings. He responded typically to exercise and to vagus stimulation. Contrary to the experiences of Frey and of Wennekebach and Winterberg,¹⁰ quinidine therapy was not followed by auricular slowing and a paradoxical ventricular tachycardia but by restitution of the normal sinus mechanism.

Because of our previously expressed conviction,²⁰ quinidine was given continuously. Normal sinus rhythm was maintained.

We are accepting without comment Bedell's analysis of reported instances of auricular flutter with 1:1 response.*

Our records are of paroxysms of 1:1 response, evoked by exercise, influenced by vagus stimulation, preceded and followed by the more typical and orthodox graphs of auricular flutter. Without this evidence, other interpretations of these unusual graphs would be possible.

CONCLUSIONS

Another instance of auricular flutter with a 1:1 ventricular response is added to those previously accepted. In our patient sinus rhythm followed quinidine administration.

REFERENCES

1. Lewis, T.: *Diseases of the Heart*, New York, 1933, The Macmillan Company, pp. 73-80.
2. Jolly, W. A., and Ritchie, W. T.: Auricular Flutter and Fibrillation, *Heart* 2: 177, 1910-11.
3. Parsonett, A. E., and Parent, Sol.: Auricular Flutter With Complete Auriculo-Ventricular Block in a Patient With Coronary Disease, *Arch. Int. Med.* 51: 938, 1933; Abstract, *Am. Heart J.* 8: 865, 1933.
4. Blackford, J. M., and Willius, F. A.: Auricular Flutter, *Arch. Int. Med.* 21: 147, 1918.
5. Bedell, Caroline, C.: Auricular Flutter With 1:1 Response, *Bull. Johns Hopkins Hosp.* 52: 225, 1933.
6. Parkinson, J., and Bedford, D. E.: The Course and Treatment of Auricular Flutter, *Quart. J. Med.* 21: 21, 1927.
7. Lewis, T.: *Clinical Disorders of the Heart Beat*, London, 1925, Shaw & Son, pp. 57, 79.
8. Mackenzie, J.: Digitalis, *Heart* 2: 379, 1910-1911.
9. Koplik, H.: Paroxysmal Tachycardia in Children, *Am. J. M. Sc.* 154: 834, 1917.
10. Scott, R. W.: A Case of Auricular Flutter With Paroxysmal Attacks of 1:1 Conduction, *J. A. M. A.* 79: 1984, 1922.
11. Wedd, A.: Clinical Auricular Flutter, *Ann. Clin. Med.* 3: 69, 1924.
12. Gallavardin, L., Bonnamour, S., and Berheim, M.: Un Cas de Flutter 1:1, *Arch. d. mal. du coeur* 17: 342, 1924.
13. MacMillan, T. M., and Sweeney, J. A.: Auricular Flutter With Periods of 1:1 Ventricular Response, *Am. J. M. Sc.* 168: 893, 1924.
14. Berard, A.: Un Cas de Flutter 1:1, *J. de Med. de Lyon* 7: 471, 1926.
15. Poynton, F. J., and Wyllie, W. G.: Auricular Flutter in Infancy, *Lancet* 2: 371, 1926.
16. Herapath, C. E. K.: Auricular Flutter, *Brit. M. J.* 1: 213, 1928.

*Wennekebach and Winterberg have also published some apparently valid instances of auricular flutter with 1:1 response.

- 17 Sprague, H B, and White, P D Auricular Flutter Report of a Case of Five Years' Duration With Spontaneous Restoration of Normal Rhythm J A M A 90 1772 1928
- 18 Fuller, A B A Case of Auricular Flutter With Paroxysmal Attacks of 1:1 Response of the Ventricles, Am Heart J 3 734, 1928
- 19 Wenckebach, K F, and Winterberg, H Die Unregelmässige Herztaetigkeit, Leipzig, 1927, W Engelmann
- 20 Carter, J B and Traut E F Quinidine and Strichnine in the Treatment of Premature Contractions Am J M Sc 189 206 1935

FLUCTUATIONS IN BASOPHILIC AGGREGATION COUNTS WITH METEOROLOGIC ALTERATIONS*

G HOWARD GOWEN, M D, CHICAGO, ILL

RED cell basophilia, although not pathognomonic of lead poisoning has long been associated with this condition and has served as a medium of diagnosis. The presence of basophilic substance has been interpreted as one of the most constant characteristics of immature red cells. McCord (1924, 1928) has devised a method of demonstrating basophilic granules which he has termed the basophilic aggregation test. The basis of this test is the hemolysis of red cells prior to staining with Manson's methylene blue. This results in an artificial aggregation of the evenly distributed basophilic substance found in young red cells and creates a greater visibility of such cells. McCord states that 66 per cent of normals may exhibit these basophilic aggregations and he sets the high normal limit at 15 per cent in his latest publication. He notes, however, that higher normal counts may occur in the newborn, during pregnancy, after arsenic or iron medications, in high altitudes, after exposure to sunlight and rays and possibly after the intake of foods. Because McCord's normal index was computed on the basis of a single estimate on each of a number of different individuals, the following experiment was devised to see whether one such count was really an indicator of a normal's basophilic aggregation level or whether fluctuations in the level might occur from day to day.

A daily study was made of blood smears from four normal laboratory workers during the month of October, 1934. Subjects I and III were females aged twenty four years and thirty years. Subjects II and IV were males aged twenty seven years and twenty two years. In no instance was there even remotely a lead hazard. Smears were made from Subjects I and II daily except Sundays between the hours of 11 00 AM and 12 00 M. Smears were made from Subjects III and IV daily except Sundays between the hours of 2 00 and 3 00 PM. The smears were stained and examined according to the McCord technique, and the total counts were made on the basis of the number of cells ex-

*From the Department of Bacteriology and Preventive Medicine University of Illinois College of Medicine Research Laboratories of the State Department of Public Health and The Chicago Medical School

Received for publication May 27 1935

hibiting basophilic aggregations per million red blood cells. At the end of thirty-one days the daily counts were charted graphically for each subject. It was immediately noticed that there were daily fluctuations and very definite peaks during the month. It was also noted that in all four cases these peaks coincided rather accurately. Because all of the subjects showed a uniformity in the type of curve, it was felt that some influence common to all had most probably produced this effect. With this in view, the four graphs were compared with a meteorograph for October. The meteorograph has been carried over Figs. 1

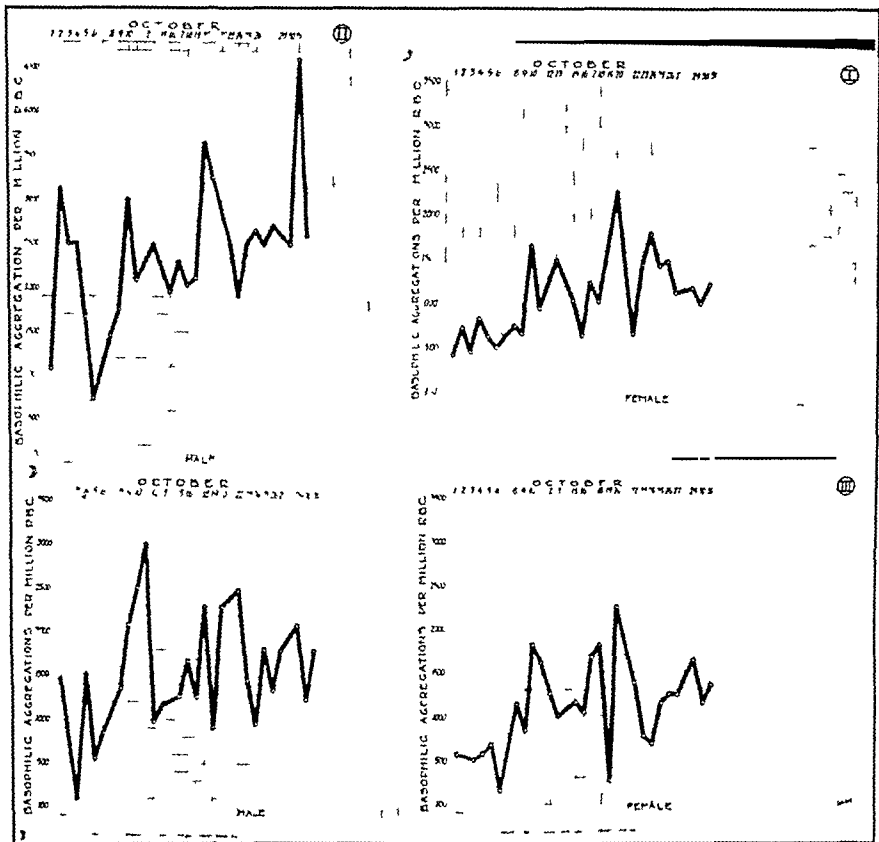


Fig. 1.—This shows the daily fluctuations and the similarity of curves in the four subjects studied

and 2, and a similar meteorograph over Figs. 3 and 4. In each case stippled vertical columns have been carried from the upper female and lower male basophilic aggregation peaks to the meteorograph above where these have been numbered one to ten. Table I indicates the meteorologic association.

The curves are alike so far as the basophilic aggregation peaks are reached, in general, on the same days and in all cases the increase in young cells is associated with the change to polar air. In Males II and IV no peak is reached on the eighth day of October with episode three as is evidenced in the females, but this effect seems to be cumulative and is carried over to reach the peak on

the tenth and eleventh with episode four. A similar cumulative action is seen in Male IV with episodes nine and ten. The peaks of Subjects II, III, and IV are deferred one day over Subject I in episodes eight and nine. The storm of the twentieth is associated with a marked rise in the curves of all four subjects. In

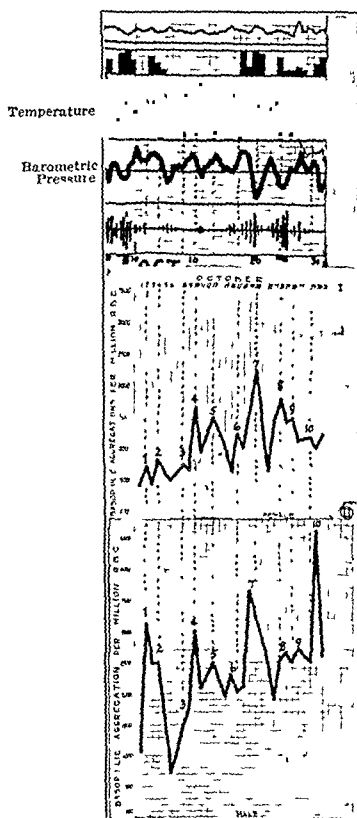


Fig 2—This shows how the basophilic aggregation peaks correspond with the high barometric and low temperature peaks in Subjects I and II

the passage from October to November and the entrance into the more disturbed season of the year, it is seen that the level of the cells exhibiting basophilic aggregations is higher than at the beginning of October. The bone marrow here reflects the effect in a moderate summation in the increased production of young cells

That blood pressor episodes (ARS phases)* are factors influencing and altering certain cellular and chemical constituents of the blood has been definitely shown by Shulruff, Schiller, Berg, Feinhandler, Milliken and Hoverson. A period of vascular spasm (ARS phase) is followed by (1) an increase in the leucocyte curve; (2) an increase in eosinophile count; (3) an increase in proportionate number of polymorphonuclear leucocytes; (4) the appearance of more young forms; (5) an increase in fibrin; (6) an increase in platelets; (7) an alteration in number of red cells; (8) an increased resistance of red blood cells

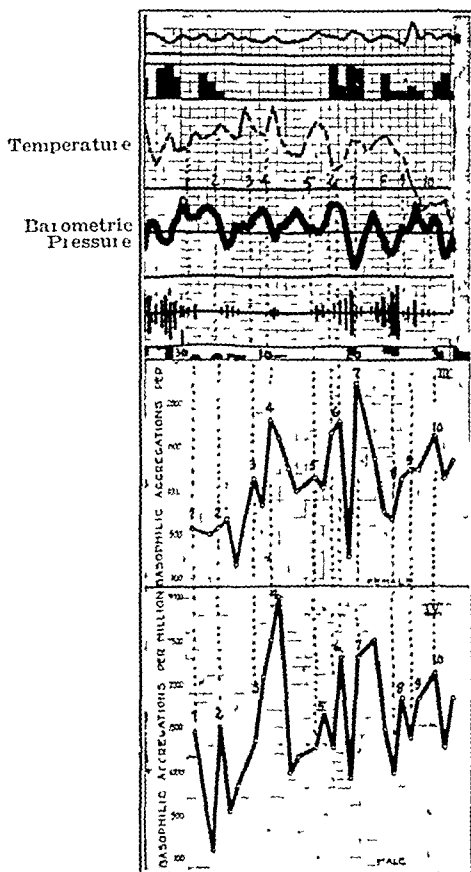


Fig. 3.—This shows how the basophilic aggregation peaks correspond with the high barometric and low temperature peaks in Subjects III and IV.

to saponin and bile; (9) variations in cholesterol content of whole blood and plasma; (10) and an increased sedimentation rate of erythrocytes. Berg also noted an increase in "stippled" cells in the dog during the ARS phase. These

*Petersen has used the abbreviations ARS and COD to designate phase alterations in the normal metabolic status of the individual responding to environmental alterations. In general, the infall of polar air is associated with an increase in the blood pressure, spasm of the vessels, anoxia, and preponderance of reduction. This stage goes over in a continuous flux to the COD phase when tissue metabolism is increased, when oxidation is enhanced, and when the tissue vessels including the capillaries are dilated. The onset of the ARS phase with its spasm of the smaller vessels is promptly associated with the stimulation of the bone marrow due to the relative anoxemia. There follows an increase in leucocytes (young forms as well as eosinophiles), in the red blood corpuscles, platelets, fibrin, etc., and a change in practically all of the serum reactions as well as in the enzymatic and chemical reactions of the body. (See Petersen: Patient and Weather—Autonomic Disintegrations, Ann Arbor, 1934, Edwards Brothers.)

effects may occur synchronously with the peak of the pressor episode or the maximum effect may be delayed until the subsequent barometric low. In Hawaii, where the meteorologic alterations are less intense than in this latitude, Fennel has shown, that there also occurs a definite increase in eosinophiles, polymorpho nuclear leucocytes, and total leucocytes, in this instance associated with changes in humidity and wind velocity.

TABLE I
OCTOBER, 1934

EPISODES	DATE OF EPISODES	DATE OF BASOPHILIC AGGREGATION PEAKS				METEOROLOGIC ASSOCIATION
		FEMALE I	MALE II	FEMALE III	MALE IV	
1	2	2	2	12	2	Polar infall begins
2	4	4	4	5	4	Polar crest
3	8	7-8	10	8	11	Polar infall
4	10	10	10	10	11	Polar infall
5	13-15	12-13	17	15	13-16	Polar infall
6	17-18	17	16	17-18	15	Polar infall (?)
7	20	20	19	20	20-22	Polar crest on 18th Storm on 20th
8	24	24	25	25	25	Polar infall
9	26	26	27	27	27-29	Polar infall
10	29	29	0	29	29	Polar infall

The results of the above experiments have a rather significant application to the lead industry. In many instances it is compulsory that a basophilic aggregation count or some other standard count be made on new employees, for the purpose of first of all determining whether they have any evidence of previous lead intoxication and second so that subsequent counts made at intervals and compared with the original would indicate whether the individual was experiencing any lead absorption. The making of the initial count or of subsequent counts without giving consideration to normal fluctuations could readily result in misleading information, depending upon whether the count was taken at a polar crest or a polar infall.

SUMMARY

1 Basophilic aggregation counts in normals are subject to rather wide daily variations.

2 In the four normals studied, these variations coincided as is evidenced by the prepared graphs.

3 The only effect in common which could have been experienced by these different individuals would have been an influence due to meteorologic alterations.

4 The daily fluctuations evidenced in these individuals are in accord with the pressor episodes resulting from influx of polar air.

5 Inasmuch as basophilus is an indicator of immature red cells, it can be assumed that an increase in such cells is an evidence of bone marrow stimulation with a liberation of young cells, and in this case is associated with the influence of polar episodes.

6 One basophilic aggregation count does not indicate the normal level for an individual inasmuch as the count may vary widely with the meteorologic changes.

7. In the preliminary basophilic aggregation counts made on individuals entering an industry where there is a lead hazard, the above facts should be taken into consideration in evaluating the results of such counts.

REFERENCES

1. McCord, C. P., Minster, D. K., and Rehm, M.: Basophilic Aggregation Test in Lead Poisoning, J. A. M. A. 82: 1759, 1924.
2. McCord, C. P.: A New Test for Industrial Lead Poisoning, Bull. U. S. Bureau of Labor Statistics, No. 460, 1928.
3. McCord, C. P.: The Basophilic Aggregation Test, Cincinnati, Ohio, 1935, Published by Industrial Health Conservancy Laboratories.
4. Petersen, W. F.: Autonomic Integration, The Patient and the Weather 1: Part 2, Ann Arbor, Mich., 1935, Published by Edwards Bros.

LYMPHOGRANULOMATOSIS (HODGKIN'S DISEASE)*

A REVIEW OF SIXTY CASES

FREDERICK J. KRUEGER, M.D., AND OVID O. MEYER, M.D., MADISON, WIS.

INTRODUCTION

SINCE the original report of Thomas Hodgkin¹ in 1832, there have been many reports of the condition which have been given his name. These have included many surveys, and various aspects of the disease have been emphasized. Because there is still little known as to the etiology and much question about symptomatology and therapy, it was deemed worth while to analyze briefly the sixty proved cases, by biopsy or autopsy, of lymphogranulomatosis admitted to the State of Wisconsin General Hospital during the decade 1924 to 1934. These sixty cases represent 0.00116 per cent of the hospital admissions.

In this study only the pertinent findings, clinical and pathological, will be discussed.

Age and Sex Incidence.—There were forty males and twenty females in all, the ratio being 2:1. All the patients were Caucasians and came from essentially rural districts. Chart 1 illustrates that the relation of the number of males in the various decades of life to the number of females is fairly uniform. The reported age is that at the onset of the disease. However, many of the cases were of as long as one to three years' duration before medical aid was sought. It is of interest that females showed the greatest incidence in the twenty-five- to thirty-four-year period, the males during the thirty-five- to forty-four-year period. No female in this series was less than fourteen years old. There were two males over eighty years of age.

Chief Complaints and Symptoms.—Swelling of the "glands" of the neck, weakness, and cough were the commonest complaints which brought the patient to the hospital. Table I, A, records the chief complaints and Table I, B, the

*From the Department of Medicine, University of Wisconsin.
Received for publication, May 29, 1935.

most common early symptoms in this series. In many patients there were several complaints, thus caused overlapping, and often, difficulty in deciding the chief complaint. Every case had an insidious onset, and these recorded complaints represent the findings after the disease had existed for a considerable period. Every patient complained of weight loss and weakness, and had noted pallor. Most of the patients complained of chills and fever, night sweats, cough, substernal pain, choking spells and anorexia. Symptoms were often dependent upon the nodes involved. Thus enlargement of the cervical, thoracic, abdominal (epigastric) or pelvic groups commonly produced distinctive symptoms as a result of pressure. Not unusually pressure on the cervical sympathetics by neighboring nodes produced inequality in the pupils and pseudoptosis of the eyelid in three instances true Horner's syndrome. Similarly, pressure by the

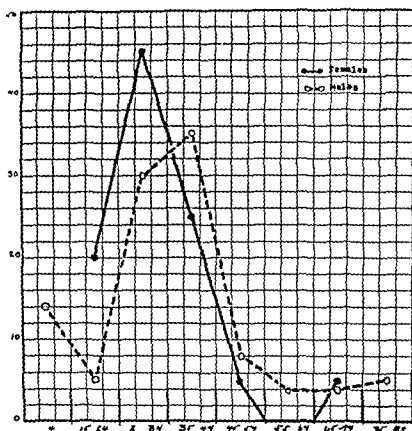


Chart 1—Percentage of males and females in various age groups for the sixty cases

intrathoracic nodes produced characteristic symptoms. Nausea, abdominal distress, hydrops of the gallbladder in two instances and other situations resulted from intraabdominal pressure, while symptoms referable to the pelvic organs, such as a feeling of fullness and low back pain, were clearly explained by the involvement of pelvic lymph nodes.

Localization Clinically and at Postmortem—Although lymphogranulomatosis is a general disease with involvement of the widespread reticuloendothelial system, there are definite areas of localization which are demonstrable clinically. These can commonly be verified at autopsy. In Table II, Column A is shown the number of cases and percentage of the sixty that clinically showed involvement of certain organs. All organs except the mediastinal nodes were definitely enlarged and palpable. The diagnosis of mediastinal node involvement and bone involvement was usually made by roentgenographic study. In

column B will be found the number of cases and percentage of the sixteen that came to autopsy, and showed gross involvement of organs. In addition there were four cases with only microscopic changes in the liver and spleen. In five cases bone marrow studies were done and disease was found in all. It is noteworthy that mediastinal node involvement was very common, demonstrable in 80 per cent of the 60 cases, and in all of the 16 which came to autopsy.

TABLE I

A. THE CHIEF COMPLAINT THAT BROUGHT SIXTY CASES OF LYMPHOGRANULOMATOSIS TO THE HOSPITAL			B. THE MOST COMMON SYMPTOMS PRIOR TO HOSPITALIZATION		
COMPLAINT	NO. OF CASES	PERCENTAGE	COMMON SYMPTOMS	NO. OF CASES	PERCENTAGE
1. Swelling of the glands of the neck	24	40.8	1. Weakness	60	100.0
2. Weakness	7	11.9	2. Weight loss	60	100.0
3. Cough	6	10.2	3. Cough	50	85.0
4. Lumps in the axilla and the groin	5	8.5	4. Fever and occasional chills	50	85.0
5. Fever	5	8.5	5. Anorexia and occasional nausea	48	81.6
6. Painful glands of the neck with choking sensations	4	6.8	6. Dyspnea and choking spells	40	68.0
7. Nausea and epigastric distress	3	5.1	7. Pain in chest	34	58.8
8. Pain in right leg	3	5.1	8. Night sweats	31	52.7
9. Backache	3	5.1	9. Nocturia (1-3x)	24	40.8
			10. Backache	20	33.3
			11. Edema	18	30.6
			12. Headaches, tinnitus, and vertigo	15	25.5

TABLE II

NUMBER OF CASES THAT SHOWED GROSS INVOLVEMENT OF CERTAIN ORGANS

A. CLINICAL INVOLVEMENT			B. POSTMORTEM	
ORGAN	NO. OF CASES	PERCENTAGE	NO. OF CASES	PERCENTAGE
Superficial nodes	58	96	14	88
Mediastinal nodes	50	85	16	100
Abdominal nodes	20	33.3	13	81
Spleen	40	66.6	12	75
Liver	55	93	12	75
Pelvic nodes	3	5	11	69
Bone (roentgenogram)	9	15	5 (micro)	31

At autopsy, the liver was grossly involved as often as the spleen, and on clinical examination the liver was palpable more often than the spleen. The palpability of the spleen in two-thirds of the cases is about the frequency usually noted in any series.

Involvement of the Special Organs Including the Skin.—In certain cases the disease was found at sites not so commonly involved. These instances were confirmed by biopsy or autopsy. There were 8 cases with infiltration into the lung tissue, 7 of these with an associated atelectasis, 3 with pleural involvement, and 2 with erosion of the bronchi. Three of the cases showed gastric and 3 pericardial involvement. The myocardium and the superior vena cava were each involved in 2 cases. There was 1 example of infiltration into each of the following organs: Thymus, sternum, right auricle of the heart, pectoral muscle, kidney, urethra, bladder, and 1 case with multiple ulcerat-

ing areas in the colon. Involvement of many of these organs could be diagnosed only at autopsy; therefore the actual incidence in the entire series can not be stated.

Gross skin manifestations were seen in 11 patients. There were, however, 14 additional patients who had pruritus without visible skin changes. These lesions were most commonly found on the exposed surfaces of the extremities and were characterized by an atrophic eczematoid chronic eruption often simulating a fungous growth. The margins usually had traumatic abrasions from scratching. Two cases showed ulceration of the skin over enlarged nodes. Increased pigmentation was found in 27 cases. This was usually characterized by dark brown molelike flat blotchy areas on exposed surfaces of the body, but a general bronzing occurred in 4 patients. Edema was usually related to lymph stasis or vascular obstruction, much less often cardiac decompensation was the cause.

Temperature, Pulse, and Blood Pressure—After the disease had established itself and was active there was fever. This was usually characterized by a remittent temperature elevation with an evening rise of two to three degrees. The average duration of the pyrexia was found to be three weeks in 54 cases and usually occurred in the latter half of the illness. The fever was usually irregular, simulating various diseases as undulant fever (2 cases) and typhoid (3 cases). Pel-Ebstein fever occurred in 1 case. The 4 patients with leucopenia usually had a subnormal morning temperature and a minimum evening temperature of 103° F. There were no patients with a continuous subnormal temperature.

The pulse was found to be rapid in every patient after the disease was well established. The rate usually varied between 100 and 120 per minute. There was a definite increase in pulse rate concomitant with the evening fever. Those patients who had an increased basal metabolic rate also had a simultaneously increased pulse rate.

Hypotension was the rule. The hypotension was not related to the extensiveness of the lesions or to the height of the daily temperature. The average systolic pressure was 110 and the diastolic pressure 74 mm Hg. When an elevated blood pressure did exist arteriosclerosis of considerable degree was present. Only one patient had marked hypertension.

Laboratory Findings—As yet there is no specific diagnostic laboratory test for lymphogranulomatosis other than microscopic study of the biopsy tissue, though the test of Gordon³ recently described may be useful. The characteristic microscopic picture on section is well known, the occasional diagnostic difficulties in study of the biopsy tissue are also well recognized and it is not our purpose to discuss these phases.

Aside from the examination of an excised node the blood findings have received the most attention. The characteristic changes in the leucocyte formula have been emphasized by Bunting.³ In these 60 cases the blood findings were not absolutely uniform. In Charts 2 and 3 will be found the blood findings in percentage of cases on admission, the average during residence in the hospital after x-ray therapy, and the last count before death. More than two thirds of the cases showed a leucocyte count above 8,000 and a majority

were between 10,000 and 25,000. The percentage of neutrophils was distinctly above normal and as the disease progressed, there was a progressive decrease in lymphocytes. The leucocytosis was not always greatest in those cases with highest fever; in fact those cases with a marked septic type of fever showed a leucopenia. There was a definite increase in the percentage of large mononuclear cells as the disease progressed. Only 5 per cent of the cases showed an eosinophilia above 5 per cent. Twenty-four (40 per cent) of the cases showed immature forms of erythrocytes. The anemia was usually moderate, the erythrocyte count being over 3,000,000 and the hemoglobin

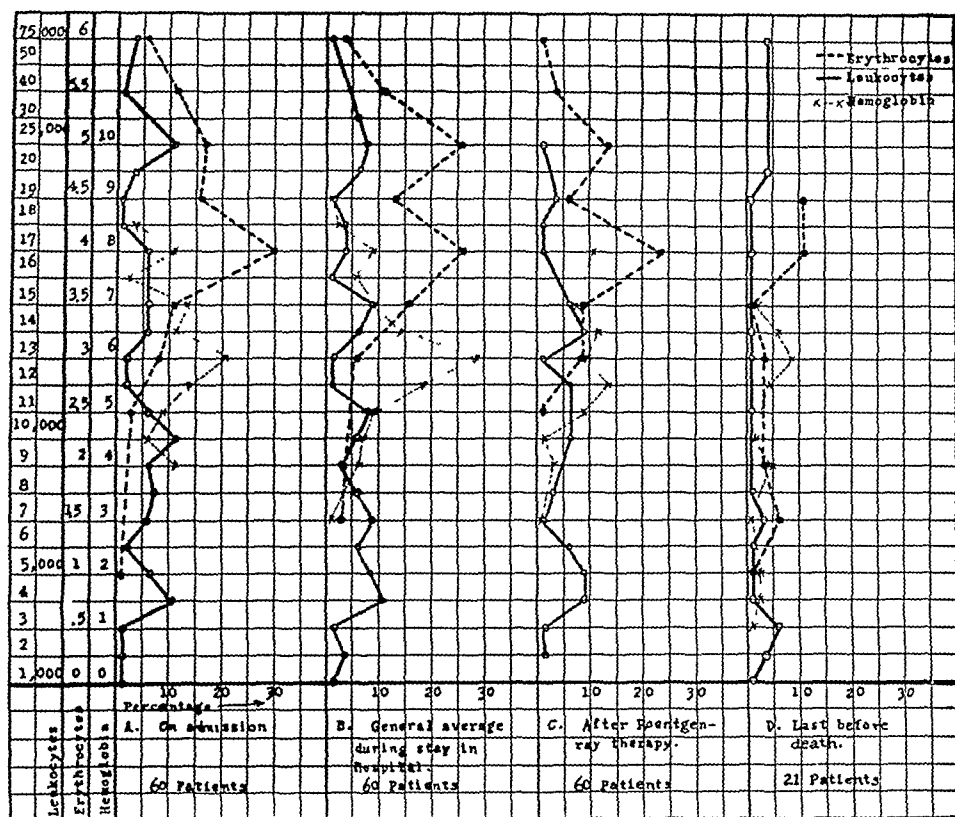


Chart 2.—The number of leucocytes, erythrocytes, and the hemoglobin at various periods of hospitalization of the sixty cases.

usually 55 to 60 per cent of normal. Of the 16 cases in which platelet counts were studied only 6 showed a moderate decrease as a deviation from the normal. All of the patients had a negative Wassermann.

The basal metabolic rate is of little value from a diagnostic standpoint but it has been noted (Krantz)⁴ that there was a definite increase in the so-called active cases. Of the 31 cases in which the metabolic rates were determined 22 showed a rate above +10 per cent. The average of the first tests for the 22 cases was +23.2 per cent.

Concerning Early Diagnosis.—An earlier diagnosis of lymphogranulomatosis is desirable, but this is frequently fraught with difficulty because of the in-

sidious onset without characteristic subjective symptoms. The common general complaints of weakness, easy fatigue, and weight loss are too common to permit suspicion of the specific disease. The additional common complaint of excessive perspiration, undoubtedly associated with an increased metabolic rate, has probably been a factor in leading to the occasional mistaken diagnosis of thyrotoxicosis.

Nearly every patient complained of cough of more or less degree, without gross evidence of pulmonary disease. The possibility of mediastinal node involvement as the cause of this symptom might suggest itself since it is very common. In several of these patients the cough was ignored or apparently explained by a goiter.

In the last analysis, the most important complaint, which is a common one that might often lead to an earlier diagnosis, is that of an enlarged node, and

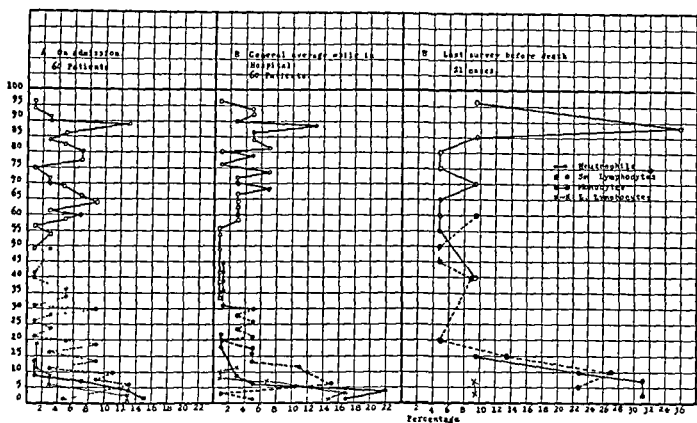


Chart 3—The percentage of neutrophils, large and small lymphocytes, and monocytes at various periods of hospitalization of the sixty cases

if early removal and microscopic examination is practiced, comparatively early diagnoses will be made. Of course, occasionally there will be no node available for biopsy.

Roentgenograms are of distinct value, especially in the detection of mediastinal involvement, in finding the cause for certain bizarre symptoms due to obstruction of a hollow viscus, and for discernment of bone involvement. Roentgenograms of the chest in these cases should be a matter of routine because of the regular involvement of the mediastinal nodes, in 100 per cent of the sixteen cases of this series that came to autopsy. Roentgenographic examination of the bones is indicated when referable complaints exist, especially those of the back since involvement of the spine is far from rare.

Prognosis—Because of the nature of the service at the Wisconsin General Hospital, where patients are referred by local physicians from various parts

of the state and then discharged to return to the care of their physicians, adequate follow-up observation is not possible. Consequently our figures as to duration of life with the disease cannot be highly accurate and no attempt is made to arrive at a prognostic figure from the cases of this series. From the patients who died in the hospital, however, it is possible to conclude that the average duration of the disease is approximately that which has been generally accepted, two to three years. Nearly every clinic has, however, observed patients who live much longer than is usual and that was true in this series. Two patients have now been under observation for more than twelve years. The diagnosis in both instances was made from biopsy of a lymph node by Dr. C. H. Bunting.

Treatment.—It is not within the scope of this paper to discuss therapeutic measures and results of therapy in detail. The treatment in all cases has taken into account the general hygienic measures and the treatment of symptoms as they arose. In recent years an especial attempt has been made to enforce the intake of a high caloric diet with a minimum of 3,000 calories, and there is a distinct impression that as a result the patients have done better than with the ordinary diet. The better results are not obtainable, however, unless a real attempt is made to cater to the patients' likes and dislikes, and to enthusiastically stimulate their interest in an abundance of food.

High voltage (180 K.V.) roentgen ray therapy has been a mainstay in most cases. All patients received it where there was no contraindication, i.e., grave uncontrolled leucopenia or anemia, a state of extremis on admission or some other contraindicating situation. In all cases, the therapy was directed to the involved sites such as the enlarged lymph nodes, the spleen, the liver, or the vertebrae. Early in the disease, satisfactory subjective and objective response to this therapy was the rule. Gain in weight and strength usually ensued, there was decrease in the size of the involved lymph nodes, spleen, and liver. This improvement was always temporary, but for variable periods of time. The lymph node enlargement recurred and the evidences of extension of the disease became more prominent. Ultimately there was no response to therapy, the lymph nodes no longer resolved, and the subsequent duration of life was invariably short. The results in this series from roentgen ray therapy tended to confirm those of Minot and Isaacs,⁵ and it seems possible to conclude as they did, that roentgen therapy makes for greater comfort for these patients, and in some cases, by relief of mechanical obstruction, considerable prolongation of life. This series is too small, and particularly is that true of the unirradiated group, to permit any conclusion as to the effect of roentgen ray therapy upon longevity in the average case.

COMMENT

Analysis of these sixty cases confirms in large part many of the established facts regarding Hodgkin's disease. The prevalence of the disease in the middle and early age groups, and the two to one predominance of the disease in males were to be expected. The high incidence of fever and tachycardia, and the frequency of an elevated basal metabolic rate are all commonly accepted as part of the disease. Likewise, the studies of Bunting.

showing the usual existence of a moderate leucocytosis with a persistent increase in large mononuclear cells and a progressive neutrophilia with a concomitant progressive lymphopenia in Hodgkin's disease, are confirmed by the majority of these cases. These findings in the differential blood picture are not universally acknowledged; nevertheless, from this series one must agree with Bunting's findings. The detail of eosinophilia in the second period of the disease was not, however, uniformly observed in these cases. This may perhaps be explained by the fact that many of the cases came to us comparatively late in their course. The great frequency of immature erythrocytes in increased numbers in the peripheral blood is noteworthy.

Analysis of the chief complaints offered by these patients brings out nothing new or striking. As might be anticipated, the chief complaint of "swelling of the glands of the neck" was the most common. Of the other complaints, however, those suggesting intrathoracic disease were very frequent. Cough, dyspnea and choking spells, chest pain, and night sweats occurred in more than half of the cases, cough being the most frequent, occurring in 83 per cent. The great frequency of these symptoms is not generally appreciated, and they tend to confirm the surprisingly high incidence of mediastinal lymphadenopathy in these sixty patients. Too much emphasis cannot be placed upon the importance of roentgenograms of the chest in all cases of Hodgkin's disease because of this high incidence and the great difficulty in establishing the existence of mediastinal involvement by physical examination alone.

The occasional unusual sites for the existence of Hodgkin's lesions in these cases emphasizes the well-known fact that practically any organ of the body may become involved.

SUMMARY

Sixty cases of Hodgkin's disease, sixteen of which came to autopsy, have been observed and an analysis has been made of the commoner subjective and objective clinical findings and the postmortem lesions. Signs referable to the respiratory system and the finding of mediastinal lymphadenopathy were surprisingly common. Hypotension, fever at some time in the disease, tachycardia and an increase in the basal metabolic rate were frequently noted. The blood findings were similar to those described by Bunting as characteristic of the disease. Marked relative and absolute lymphopenia were common as the disease progressed.

High voltage roentgen therapy relieved symptoms in most of the treated cases. This series did not permit conclusions relative to prolongation of life as a result of irradiation except in rare instances when the therapy brought about diminution in the size of nodes that were producing mechanical obstruction which appeared to be incompatible with life.

REFERENCES

1. Hodgkin, Thomas: *J Med Chir Soc London* 17: 68, 1832
2. Gordon, M. H.: Remarks on Hodgkin's Disease, *Brit. M. J.* 1: 641, 1933
3. Bunting, C. H.: Hodgkin's Disease, *Johns Hopkins Hosp. Bull.* 22: 369, 1911. *The Blood Picture in Hodgkin's Disease* (second paper), *Ibid.* 25: 1914
4. Krantz, C. I.: The Basal Metabolism in Lymphoblastoma, *Am. J. M. Sc.* 176: 577, 1928
5. Minot, G. R., and Ivers, R.: Lymphoblastoma, *J. A. M. A.* 86: 1185 and 1265, 1926

THE RESPONSE OF BLOOD UREA NITROGEN, URIC ACID AND PLASMA CHOLESTEROL TO PARENTERAL LIVER EXTRACT*

DAVID SCHEINBERG, M.D., PHILADELPHIA, PA.

DURING the course of diffuse glomerulonephritis one finds renal decompensation, either temporary or permanent. In the progression of events (see Chart 1) such occasions of decompensation (azotemia) are observed in the acute stage, also in the severe subacute type and in the end stage. Azotemia may occur also when an acute exacerbation is superimposed on chronic nephritis and when chronic nephritis is complicated by an acute infection or cardiac decompensation.

The treatment of diffuse glomerulonephritis is as yet symptomatic, and will probably remain so until the causative agent is discovered. When the kidneys fail to rid the body of the end-products of protein metabolism, other organs assist in excretion. Thus the uriniferous breath, the uremic frost, the diarrhea are all signs of the attempt by the salivary glands, the skin and the bowel, respectively, to help out in this emergency. Pucher and Sly¹³ found an increase in urea nitrogen in the gallbladder bile of patients dying with urea nitrogen retention in the blood. They studied patients with pneumonia, peritonitis and nephritis in whom there was an increase in blood urea nitrogen. They observed that the composition of the blood has a pronounced effect on the amounts of the nonprotein nitrogen constituents found in gallbladder bile.

Our Problem.—Using concentrated liver extract parenterally, we desired to promote extrarenal excretion of urea nitrogen. A stimulant that would increase the liver's excretory power has a place in the treatment of azotemia. Dr. Richard Bauer,² Professor of Medicine, University of Vienna, reported on the use of parenteral liver extract in treatment of coma due to liver insufficiency. Perhaps parenteral liver may augment the liver's excretory capacity. We studied the effect of parenteral liver on blood urea nitrogen, uric acid, and plasma cholesterol. The latter was included because of clinical evidence of a reciprocal relation between the blood urea and plasma cholesterol.

It is common knowledge that as chronic nephritis progresses toward the end stage, cholesterol diminishes as the urea nitrogen rises (see Chart 1). As more and more glomeruli become completely obliterated by disease, they no longer are the site at which proteins are lost from the blood into the urine. The defective filtration area is greatly reduced, protein loss is diminished, and the blood proteins gradually tend to return to normal. With this increase in blood

*From the Medical Services of the Jewish Hospital of Philadelphia.

Received for publication, June 4, 1935.

Thesis submitted to the Faculty of the Graduate School of Medicine of the University of Pennsylvania in partial fulfillment of the requirements for the degree of Master of Medical Science (M. Sc. (Med.)) for graduate work in Internal Medicine.

proteins (oncotic pressure), plasma cholesterol decreases. Bruger and Poin dexter⁵ observed a diminution of plasma cholesterol upon the ingestion of urea. They considered this result as possibly due to increased osmotic pressure exerted by the urea.

As urea increases in the blood, urea excretion by the liver increases.¹³ Perhaps this mode of extrarenal urea elimination brings about a concomitant excretion of plasma cholesterol by the bile. Having this possibility in mind, we studied the effect of parenteral liver on urea nitrogen and cholesterol.

Selection of Patients—The studies were carried out on patients in the wards of the Jewish Hospital. The patients, white males and females, ranged in age

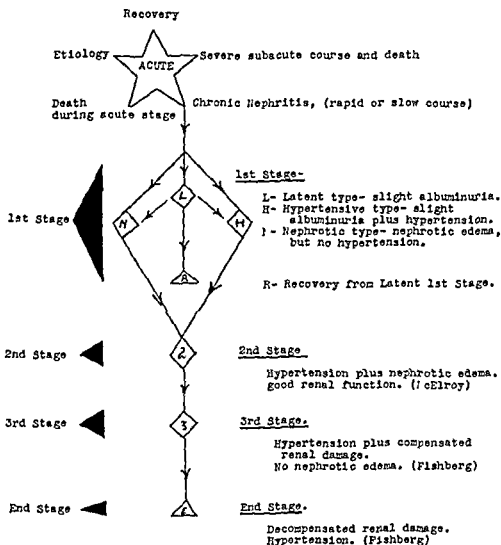


Chart 1—Diagram of the succession of events in diffuse glomerulonephritis. Bright's disease may make its first appearance clinically as chronic diffuse glomerulonephritis the patient having been unaware of an acute attack.

between twenty four and seventy five. Each one selected had been in the wards at least three days prior to the beginning of studies. Patients with biliary obstruction were excluded. Hawkins and Wright⁶ have observed hypercholesteremia in such cases. Tindal¹⁴ observed a prolonged blood nonprotein nitrogen curve upon feeding urea to children suffering from various forms of hepatic disease. Patients suspected of having hepatic disease were therefore not included.

Method of Study—The first fourteen patients, Group A, were studied as follows. First day at 8:30 A.M. blood for urea nitrogen, uric acid, plasma cholesterol determinations, at 10:00 A.M. 6 c.c. concentrated liver extract (Lilly or Lederle G fraction of Cohn) intramuscularly at 2:30 P.M. one half ounce

of a 50 per cent solution of magnesium sulphate. Second day: at 6:30 A.M. one-half ounce of magnesium sulphate; at 8:30 A.M. blood for urea nitrogen, uric acid, and plasma cholesterol; at 10:00 A.M. 6 c.c. liver extract intramuscularly; at 2:30 P.M. magnesium sulphate. Third day: at 6:30 A.M. magnesium

TABLE I
GROUP A. LIVER EXTRACT PLUS MAGNESIUM SULPHATE

PATIENT	DIAGNOSIS	DAY	UREA N. MG.	URIC ACID MG.	CHOLESTEROL MG.	ALBUMIN GM.	GLOBULIN GM.
1	Chronic nephritis	First	121	13.7	312	2.9	0.9
		Second	114	15.6	314		
		Third	127	12.5	312		
2	Chronic nephritis	First	65	8.6	227	3.3	1.7
		Second	60	8.4	238		
		Third	69	8.9	200		
3	Chronic nephritis	First	61	10.7	290	3.2	1.1
		Second	60	11.7	337		
		Third	58	11.0	294		
4	Nephrosclerosis	First	54	7.5	263	5.2	1.7
		Second	55	10.0	200		
		Third	55	8.7	166		
5	Chronic nephritis	First	41	8.2	196	4.6	1.4
		Second	41	8.7	150		
		Third	41	8.5	192		
6	Nephrosclerosis	First	49	4.8	156	4.2	2.1
		Second	40	6.0	130		
		Third	35	5.0	135		
7	Nephrosclerosis	First	20	3.5	140	4.6	2.4
		Second	23	6.0	139		
		Third	23	4.2	140		
8	Nephrosclerosis	First	17	2.6	208	5.1	1.8
		Second	10	2.1	166		
		Third	20	3.0	165		
9	Diabetes	First	17	5.0	166	5.1	2.0
		Second	16	4.8	207		
		Third	18	5.2	277		
10	Diabetes	First	28	8.0	200	4.0	1.2
		Second	23	9.0	243		
		Third	20	8.2	227		
11	Diabetes	First	23	4.6	279	5.1	1.3
		Second	20	3.7	227		
		Third	17	5.2	249		
12	Diabetes. Pituitary dysfunction	First	18	5.2	147	5.5	0.5
		Second	23	4.7	138		
		Third	20	4.1	140		
13	Diabetes	First	26	3.0	208	4.6	1.9
		Second	16	3.2	156		
		Third	18	3.2	192		
14	Diabetes	First	18	3.7	238	4.2	2.8
		Second	18	3.8	192		
		Third	15	3.5	178		

sulphate, at 8:30 A.M. blood for urea nitrogen, uric acid, and plasma cholesterol. The magnesium sulphate was given to promote gallbladder emptying. The patients experienced about three bowel movements a day.

The next ten patients, Group B, were studied for the effect of liver extract on cholesterol alone. These did not receive magnesium sulphate or any other drastic cathartic. Blood was collected for plasma cholesterol determinations on three consecutive days at 8:30 A.M. At ten o'clock of the first and second mornings the patients received 6 cc. of liver extract intramuscularly.

Group C consists of two patients who received 3 cc. of liver extract intramuscularly for twelve successive days. Plasma cholesterol determinations were made before and after.

In Group D are three patients who received magnesium sulphate alone. The time of administration of the salt and collection of blood for plasma cholesterol was the same as that in Group A.

TABLE II
GROUP B LIVER EXTRACT—CHOLESTEROL

PATIENT	DIAGNOSIS	DAY	CHOLESTEROL MG	ALBU- MIN GM	GLOBU- LIN GM	SUM MG	DAILY INSULIN
1	Diabetes	First Second Third	214 217 200	4.5	2.5	192	
2	Diabetes	First Second Third	192 200 208	4.6	1.4	168	45
3	Diabetes	First Second Third	262 200 217	4.1	1.9	206	40
4	Diabetes	First Second Third	172 200 192	4.4	1.6	174	50
5	Diabetes	First Second Third	249 192 208	4.2	2.8	150	30
6	Nephrosclerosis	First Second Third	186 262 249	5.6	1.2	86	
7	Nephrosclerosis	First Second Third	178 192 178	6.5	1.9	81	
8	Chronic nephritis	First Second Third	227 230 200	3.3	1.7	118	
9	Parkinsonism	First Second Third	156 166 157	5.6	5.6	114	
10	Convalescent pneumonia	First Second Third	189 172 178	6.5	1.7	130	

The blood cholesterol of three patients, Group E, were studied on three consecutive mornings. These received neither liver extract nor magnesium sulphate.

Such drugs as digitalis, insulin, and phenobarbital which the patients received before the experiment was begun, were allowed during the experiment. No drug was introduced during the period of study. Serum albumin-globulin determinations were made on all the patients. All blood studies were performed on fasting subjects by the same technician in the Laboratories of the Jewish Hospital. The following methods were used: Marshall's Modified Aeration for urea nitrogen, Folin-Wu for uric acid, Bloor for cholesterol, Folin-Wu and Micro-Kjeldahl for serum albumin and globulin. The blood chemistry quantities are recorded for 100 c.c. of blood.

TABLE III
GROUP C. LIVER EXTRACT, 3 C.C. DAILY FOR TWELVE DAYS

PATIENT	DIAGNOSIS	DAY	CHOLESTEROL MG.	ALBUMIN GM.	GLOBULIN GM.	SUGAR MG.	DAILY INSULIN
1	Pernicious anemia	First Thirteenth	198 149	4.6	1.2	98	
2	Anemia; diabetes	First Thirteenth	248 210	5.1	1.3	196	55

TABLE IV
GROUP D. MAGNESIUM SULPHATE

PATIENT	DIAGNOSIS	DAY	UREA N. MG.	URIC ACID MG.	CHOLESTEROL MG.	ALBUMIN GM.	GLOBULIN GM.
1	Diabetes	First Second Third	23 20 29	3.0 4.5 3.7	178 217 208	4.6	1.9
2	Diabetes	First Second Third	20 23 23	3.8 5.7 5.5	238 192 227	4.2	2.8
3	Nephrosclerosis	First Second Third	16 15 18	4.1 3.4 4.1	198 192 217	5.5	1.2

TABLE V
GROUP E. NO MEDICATION

PATIENT	DIAGNOSIS	DAY	CHOLESTEROL MG.	ALBUMIN GM.	GLOBULIN GM.	SUGAR MG.	DAILY INSULIN
1	Diabetes	First Second Third	217 190 180	4.1	1.9	200	40
2	Chronic nephritis	First Second Third	300 273 249	3.3	1.7	118	
3	Diabetes	First Second Third	277 279 247	4.2	2.8	138	35

COMMENTS AND OBSERVATIONS

Each patient was on practically the same diet before and during the experiment, rigid control of diet was not attempted. Bruger and Ponder^{3, 5} observed no immediate change in plasma cholesterol values following the ingestion of water and fat. No immediate response in plasma cholesterol was observed by Bruger and Mosenthal⁴ following the injection of insulin and epinephrine. Bruger and Somach⁶ obtained a daily variation of 8 per cent plus or minus in the plasma cholesterol of normal subjects. In our three patients (Group E) the following variations were obtained: in the first patient, plus 14 per cent and minus 5 per cent, in the second, plus 9 per cent and minus 8.8 per cent, and in the third, plus 0 per cent and minus 10 per cent.

Gebhardt and Klem⁷ observed an increase in plasma cholesterol of nineteen patients following intramuscular injections of liver extract. They administered 3 cc of the extract daily for a period of eight to twenty-two days. The increase was occasionally as high as 50 mg. In our two patients, Group C, we observed a decrease of 49 mg in one, and 38 mg in the other.

In studying the response of plasma cholesterol to liver extract in Groups A and B, one finds no consistent results if he considers all patients alike. However if the diabetic patients are separated from the nephrotic patients and the nephrosclerotic patients, one observes the following. In all the diabetic patients of Groups A and B, whose plasma cholesterol was above 200 mg on the first day, a decrease is seen on the second day (initial fall). Thus in Cases 11, 13, and 14 of A the plasma cholesterol diminished 18.5 per cent, 25 per cent, and 19 per cent, respectively, following the first injection. Cases 1, 3, and 5 of B had a decrease of 22 per cent, 23 per cent, and 23 per cent, respectively. In all the other diabetic patients the plasma cholesterol on the first day was 200 mg or less. All of these, except Case 12A, showed a rise in cholesterol. (Case 12A is one of pituitary dysfunction; the diabetes may be secondary to the pituitary disturbance.) The increase in cholesterol was 4 per cent, 16 per cent, and 26 per cent in Cases 9A, 2, and 4 of B, respectively.

Among the nephrosclerotic patients there were two patients whose plasma cholesterol on the first day was above 200 mg. Both of these showed a fall in cholesterol following the first injection of liver (Cases 4 and 8 in A). However only one case (6B) of the remaining nephrosclerotic patients whose plasma cholesterol was 200 mg or less revealed a rise. In this respect the response in the nephrosclerotic patients differed from that of the diabetic patients.

In some of the cases which showed a decrease of plasma cholesterol following the first injection, a slight rise was noted following the second. This however was not uniform.

The response of cholesterol in diabetic patients to liver alone and to liver plus magnesium sulphate is apparently the same as that to magnesium sulphate alone. In Group D (response to magnesium sulphate) there are two diabetic patients, one with a cholesterol above 200 mg. These two behaved exactly as the diabetic patients in the other groups. The one with 178 mg increased to 217 mg (22 per cent), the one with 238 mg decreased to 192 mg (19 per cent).

The serum protein level is probably one of the factors concerned with the

response of cholesterol to liver and to magnesium sulphate. Four of the five nephritic patients had a low total serum protein (Cases 1, 2, 3 in A and 8B). The initial fall in cholesterol was not observed in these although the cholesterol was above 200 mg. While in Case 5A with a total serum protein of 6 gm. the cholesterol decreased from 196 mg. to 150 mg. (23 per cent).

The response of blood urea nitrogen and uric acid to liver and magnesium sulphate was neither constant nor significant.

SUMMARY AND CONCLUSIONS

1. The effect of parenteral liver extract on blood urea nitrogen, uric acid, and plasma cholesterol was studied.
2. No significant response in urea nitrogen and uric acid was observed.
3. The response of plasma cholesterol in the diabetic patients was uniform. (a) Diabetic patients with plasma cholesterol *above* 200 mg. showed a *decrease* following the first injection. (b) Diabetic patients with plasma cholesterol of 200 mg. or less showed an *increase* following the first injection.
4. The change (initial rise and fall) in the plasma cholesterol level obtained with parenteral liver alone is apparently the same as that obtained with magnesium sulphate alone.
5. Serum protein is one of the factors governing the response of plasma cholesterol to parenteral liver.
6. We did not observe an increase in plasma cholesterol following liver injections daily for twelve days as had been previously reported by another writer.⁷

We are most appreciative for the cooperation of the Jewish Hospital and the Jewish Hospital medical staff. We are indebted to Dr. Edward W. Steinfeld and Mrs. Carrie B. Nash for their help in the blood chemistry studies.

REFERENCES

1. Ashe, B. I., and Bruger, Maurice: The Cholesterol Content of the Plasma in Chronic Nephritis and Retention Uremia, *Am. J. M. Sc.* 186: 670, 1933.
2. Bauer, Richard: A New Treatment for Various Kinds of Coma, *Ann. Int. Med.* 8: 595, 1934.
3. Bruger, Maurice, and Poindexter, C. A.: Relation of the Plasma Cholesterol to Obesity and to Some of the Complicating Degenerative Diseases, *Arch. Int. Med.* 53: 423, 1934.
4. Bruger, Maurice, and Mosenthal, Herman O.: The Immediate Response of the Plasma Cholesterol to the Injection of Insulin and Epinephrine in Human Subjects, *J. Clin. Investigation* 13: 399, 1934.
5. Bruger, M., and Poindexter, C. A.: The Effect of the Ingestion of Water and of Urea on the Cholesterol Content of the Plasma, *J. Biol. Chem.* 101: 21, 1933.
6. Bruger, M., and Somach, I.: The Diurnal Variations of the Cholesterol Content of the Blood, *J. Biol. Chem.* 97: 23, 1932.
7. Gebhardt, F., and Klein, J.: The Effect of Liver Extract on Cholesterol and Cholesterol Esters in the Blood, *Klin. Wchnschr.* 12: 494, 1933.
8. Harding, V. J., and Myers, V. C.: Non-protein Nitrogen Constituents of the Blood, *The Cyclopedia of Medicine*, Philadelphia, F. A. Davis Co.
9. Hawkins, H. B., and Wright, A.: Blood Plasma Cholesterol, Fluctuations Due to Liver Injury and Bile Duct Obstruction, *J. Exper. Med.* 59: 427, 1934.
10. Howell, W. H.: Blood Urea Nitrogen, *A Text Book of Physiology*, Philadelphia, 1933, W. B. Saunders Co.
11. Macleod, J. J. R.: Uric Acid in the Blood; Physiology and Biochemistry in Modern Medicine, St. Louis, 1930, The C. V. Mosby Company.

- 12 Marifón, G, Collazo, J, Gutin, L and Roda E The Action of the Adrenal Cortical Hormone on the Elimination of Cholesterol by Bile, *Endocrinology* 18 393, 1934
- 13 Pacher, G W, and Sly, G E A Comparative Chemical Analysis of Fistula Bile and Gall Bladder Bile for Sugar and Non protein Nitrogen Fraction, *Buffalo General Hosp Bull* 1928
- 14 Sperry, W M Lipid Excretion, *J Biol Chem* 71 351, 1927
- 15 Tindal, M T The Non protein Nitrogen of the Blood in Health and in Hepatic Disease, *Arch Dis Child* 8 17, 1932
- 16 Trumper, M, and Cantow, A Urea, Uric Acid, Cholesterol, *Biochem Int Med, Philadelphia*, 1933, W B Saunders Co

AUTOHEMAGGLUTININ*

WILLIAM P BELK, M.D., F.A.C.P., ARDMORE, PA

THERE is a lack of appreciation of the fact that autohemagglutinin is not of uncommon occurrence in human blood, and that it almost surely has a definite relation to posttransfusion reactions. The following case is instructive.

The patient was a girl of nine years in the twelfth day of a typical attack of lobar pneumonia. On this day an extension to an additional lobe had occurred. No sputum could be obtained for bacteriologic study. Blood culture had been sterile. Transfusion was recommended.

The patient was blood Type A. Clot of both patient and in A donor were warmed to 37° C just before separation of the serum. Cells were washed twice in warm saline. On microslides the patient's serum was found to agglutinate her own cells, and those of the donor almost completely in thirty minutes at 20° C and at 9° C. The same combinations of serum and cells placed at 37° C for thirty minutes in a moist chamber showed no agglutination at all. The patient's serum likewise agglutinated the cells of an O blood (unwashed) at 20° C. The donor's serum showed no agglutination in any case.

Five tenths of a cubic centimeter of the patient's serum and 0.5 cc of the patient's packed washed cells were placed at 2° C for thirty minutes and shaken several times. The tube was centrifuged while surrounded with ice water, and the serum removed at once. This serum showed only a trace of agglutination of the patient's cells, the Type A donor's cells and Type O cells at 9° C, after thirty minutes. The agglutinin had been absorbed almost completely.

The patient's serum, unheated, was progressively diluted and mixed with equal amounts (0.5 cc) of a 1 per cent suspension of the patient's cells and of the cells of the Type A donor. After thirty minutes at 9° C agglutination was visible to the eye in dilutions up to and including 1:16 in both series. Drops removed and observed with the microscope showed agglutination up to and including dilution 1:64. These same tubes were then placed at 7° C for thirty minutes, after which all agglutination had disappeared as observed with the microscope. There was no hemolysis.

The clumping produced by the patient's serum when mixed with her own and with the donor's cells had an appearance typical of isoagglutination. A few rouleaux, however, were seen. To both these mixtures lecithin as a rather heavy saline suspension was added. After thirty minutes at 20° C there was disappearance of the rouleaux, but only slight breaking up of the clumps of cells. The majority of the clumps persisted.

*Instructor in Clinical Biochemistry, Graduate School of Medicine, University of Pennsylvania.

This blood, then, contained an autohemagglutinin of 1:64 titer, typical in that it was sensitive to changes in temperature, reversible, absorbable, and not appreciably affected by lecithin.

Transfusion was not attempted.

The autohemagglutinin, unless of extremely high titer, is not detected by the customary technic, which makes use of chilled clots and room temperature incubation of the serum-cell mixtures.

TIMES MEDICAL BUILDING

MEDICAL ASPECTS OF PERIODONTOCLASIA AND GINGIVITIS*

JAMES C. HEALY, M.D., FRANCIS H. DALEY, D.M.D., AND MARIAN H. SWEET, A.B.,
BOSTON, MASS.

PERIODONTOCLASIA—pyorrhea alveolaris, interstitial gingivitis, or Rigg's disease—is one of the oldest oral diseases peculiar to man. Its incidence has been noted in skulls reputed to be more than thirty thousand years old.

In the second book of Hippocrates there is a description of an oral condition in which the gums are unhealthy and the teeth loosened. The objective findings of Hippocrates coincide with the signs of our present-day prevalent pyorrhea. In modern times medicine and dentistry as professions have recognized the universality of the disease and have instituted a vast amount of laboratory and clinical research concerning its pathogenesis and therapeutics.

The literature of this subject is extensive and conflicting. Contradictory definitions and classifications render the majority of the articles almost valueless when they are studied critically. From an internist's viewpoint, and in view of the material to be presented in this communication, the most satisfactory definition found was that of Delater:¹ "Pyorrhea is a chronic destructive complication of a latent gingivitis."

In a study of periodontoclasia and its precursory gingivitis the physician and dentist have two important objectives. First, there is the consideration of the probable deleterious effects of paradental suppuration on general health. This doctrine of focal infection is generally recognized and appreciated by both branches of the healing art. Everyone grants that occasionally a diseased tooth or its supporting structures may be the source of joint, nerve, muscle, or eye disease. Nevertheless, any series of cases of arthritis or other chronic disease will yield a disappointingly large percentage of therapeutic failures if sole dependence is placed upon exodontia. The multitude of edentulous young and middle-aged sufferers of chronic disease attest the enthusiasm or despair of those who emphasize the rôle of dental and paradental disorders in systemic disease.

The second objective is that of etiology.

The authors group the conditions, gingivitis and periodontoclasia, because of their identical underlying etiology. The difference represents the time factor

*Received for publication, June 27, 1935.

or duration and the extension of the process from mucous membrane to bone or vice versa. To be sure, certain cases of gingivitis are acute. Some of these are of bacterial origin and are cured by proper local treatment. The majority of the acute and practically all of the subacute and chronic cases, particularly latent involvements of the alveolar gingivae, are not of the infectious type, however. In these nonparasitic forms, the pathologic process develops in such a way as to interfere with the integrity of the periodontal structures. The occurrence of subclinical gingivitis is not uncommon and may be overlooked until dental roentgenographs are studied. Frequently, radiographic studies of the alveolar bone have demonstrated extensive resorption in individuals whose mouths were reported to be healthy. Later, carefully taken histories and re-examinations revealed information consistent with Delater's description of the disease.

Etiology—A study of alveolar gingivitis and its end result, periodontoclasia, resolves itself into an evaluation of the local etiologic factors and the systemic disturbances which impair the nutrition of the gums and tooth supporting structures. Since all these tissues are constantly traumatized, the process of cell rebuilding must equal that of degeneration. Where nutrition is impaired or curtailed, the catabolic phase is dominant. In periodontoclasia the picture is that of degeneration or failure of regeneration.

In our work at the Tufts College Dental School Clinic, emphasis has been placed on the circulation of the gingivae and deeper structures. In this region there is an enormous capillary bed which is constantly undergoing change in response to physiologic stimuli. The arteries supplying this capillary field are innervated and the arterioles are subject to dilating and constricting influences. Disturbances of the vagosympathetic system therefore improve or embarrass the paradental blood supply. Arteriosclerosis, general or local, lessens the flexibility of gingival and adjacent tissue circulation. This sclerosis may be the sole cause, a complicating factor, or the end result of the common pathogenesis of periodontoclasia.

Other conditions which are analogous to vascular changes, in that they deplete the tissues, are the blood dyscrasias. Depending upon the severity of the disease, the oxygen and nutritional supply are lessened and there is interference with the waste removal.

Actually, in this series of cases, the authors have found that the circulatory disturbances were the chief etiologic factors.

In the six-year study of 5,446 mouths for Vincent's infection, one of the authors⁷ has reached the conclusion that most cases of gingivitis have some etiologic factor which is intimately concerned with systemic dysfunction. He stressed this fact both in acute and chronic types of the condition, and attempted to consider all possible local and general factors. It appeared, however, from the preliminary work presented in that communication that a means of diagnosis and treatment of many of our "failure" and "chronic" cases, both gingivitis and periodontoclasia, might be developed.

The chief difficulty in our approach to the study of these conditions was the necessity of a single diagnostic procedure which would aid in classifying these patients. Eventually it was discovered that the differential leucocyte count

of blood obtained from the gingivae would serve this purpose. The routine examination of gingival blood served to divide the patients into two groups, those in whom the gingival smears were identical with ear or finger tip capillary blood smears; and second, those in whom there was a local oral eosinophilia. Among the first group were found those patients whose cell nutrition was impaired by organic disease. Arteriosclerosis was the most common disease noted. This was a part of the picture of *diabetes mellitus* in four cases but in the majority it was related to the extensive vascular degenerative process ordinarily manifested by changes in the brain, kidney, and heart. Disturbances of cholesterol balance and endocrinopathies through their effect on the autonomic nervous system were thought to be the etiologic factors in four cases.

Allergic Nature.—In man, the common alterations of tissue function resulting from contact with a protein to which the individual is hypersensitive are: spasm of smooth muscle, spasm of arterioles and arteries, dilatation of capillaries, increased capillary permeability, and local increase of tissue fluids.³ The allergic response is not universal but may be strictly confined to one organ or even part of an organ. In bronchial asthma, for instance, there is spasm of the bronchiolar muscle; in gastrointestinal food hypersensitiveness there is spasm of the intestinal musculature and, in some forms of angina and myocardial conduction disturbances, there is probably a spasm of the coronary arteriole.⁴ The manifestations of allergy may be acute, remittent, and chronic.^{5, 6}

Aware of the fact that the allergic diathesis is a congenital biologic alteration of the living cell which changes the capacity of the organism to react to infection and intoxication, it seemed reasonable at least to presume that there might be an allergic factor in the genesis of some oral conditions. Furthermore, the vast majority of proteins which come in contact with the cells of the body enter through the mouth.

The recognized syndromes due to protein hypersensitiveness have multiplied in the past few years. When hay fever and asthma were thought of as the only allergic diseases, it was stated that approximately 10 per cent of the population had an inherited idiosyncrasy of this sort. As other functional disturbances were investigated the morbidity rate of allergic disease rose and in the light of present research one is led to speculate that the constitutional make-up of almost all predisposes to vulnerability of certain tissues to protein intoxication. In addition, the luxuries of civilization, the variety of diet, the extensive cuisine of our modern life, and finally the frequency of acute infections with their consequent bacterial allergy or hyperergy multiply enormously the possibilities of activating a latent tendency to hypersensitiveness.

The study included 86 cases of periodontoclasia, 27 cases of acute, sub-acute, and chronic gingivitis, and 10 cases of that form of gingivitis commonly found in the latter months of pregnancy.

Investigation of the history of these patients revealed the incidence of some form of allergic disease, actual or presumed, in 46 of the periodontoclasia cases and 16 of the gingivitis cases. The occurrence of asthma, hay fever, migraine, urticaria, serum sickness, or infantile eczema in the patient's or a relative's past life was considered to be actual evidence of an allergic diathesis. A his-

tory of vague gastrointestinal disturbances unexplainable by the ordinary diagnostic procedures was interpreted as a probable sign of protein hypersensitivity.

History and examination revealed the facts that cancrum oris was present in or had been experienced frequently by 34 of the periodontoelasia patients and 21 of the gingivitis cases.

The entire group had friable gingivae, a tendency of the gums to bleed easily when traumatized, and in the gingivitis series local discomfort varied from tenderness to severe pain in some cases.

Each patient was instructed to clean the mouth with castile soap and dry with absorbent cotton. Then the free margin of the gingivae were stimulated with a scaling instrument and when bleeding was free, blood was transferred directly to and smeared on a glass slide. The preparations were fixed by heat and stained with Wright's methylene blue eosin preparation.

Local gingival eosinophilia was detected in 74 of the 86 cases of periodontoelasia and in each case of the gingivitis group. A study of the oral bacterial flora was inconsequential in the periodontoelasia group. In 6 cases of gingivitis, smears were typical for acute Vincent's infection. In 3 of these patients the gingivitis preceded the infection of Vincent organisms by four days.

In addition to the gingival eosinophilia another finding of consequence in establishing an allergic etiology was the relatively low incidence of polymorphonuclear neutrophils and the preponderance of the nongranular white corpuscles in the blood smears from these cases. Specimens of blood taken from ear or finger puncture wound were found to be normal and did not reveal the variations of the several types enumerated above. The remaining 13 cases of periodontoelasia had normal leucocyte counts by both techniques.

A control group of 35 patients who were afflicted with allergic asthma, hay fever, or urticaria were studied for the incidence of gingival eosinophilia. Each patient had normal gums and periodontal structures. In no instance did the differential leucocyte count of the gingival blood vary from that of blood obtained by skin puncture.

A second group of 20 controls young healthy nonallergic individuals, failed to produce a single gingival eosinophilia.

The minimal local pathologic signs in the 27 gingival inflammations were swelling and redness of the margin and interdental papillae. This extended over the lingual and buccal surfaces in 20 to the buccal mucosa in 8, to the sublingual mucosa in 6 to the palatal mucosa in 7, and to the anterior pillars of the fauces in 1 case. Ulcers of sizes and severity comparable to the degree of the gingivitis were present somewhere on the inflamed surface in 9 of these cases. The breath was foul in each instance.

Six cases of this 27 patient group were termed chronic gingivitis for the duration of the condition was over six months in each instance. There was congestion and hypertrophy of the interdental papillae and calculus was present. The latter was the ordinary silvery variety in one case, and the serumal or sanguinary form which is associated with periodontoelasia in a second case.

The entire group of 101 patients were subjected to skin tests. The indentation technic was followed and approximately 120 tests for protein sensitization were performed on each individual. The diagnostic allergens included foods, animal emanations, clothing materials, bacterial proteins, and such incidentals as tobacco and dusts.

Eighty-three of the 101 patients responded in allergic fashion to one or more foreign proteins, the vast majority having several idiosyncrasies. Fifteen of the remaining allergic cases had positive skin reactions to a secondary proteose isolated from the individual's urine. The remaining 3 cases, all of which were affected by acute gingivitis, were studied by means of Rowe's⁷ elimination diets.

The proteins to which the patients in these groups reacted allergically covered the entire range of foods, emanations, and incidental proteins. Fifty-six per cent of the allergic group had positive tests to one or more of the proteins of wheat, milk, and egg. Thirty per cent were hypersensitive to tobacco. Four patients who were improved by withdrawal of tobacco were cigaret smokers. In view of this finding, and cognizant of the fact that tobacco sensitiveness is not the result of nicotine contact, ten pipe smokers were examined for gingival eosinophilia. In none did it occur.

Three cigaret smokers who were hypersensitive to tobacco were advised to use holders. Five days later, even though the same number of cigarets were being consumed daily, the gingival blood was found to be normal. On this scanty study, it might be assumed that the cigaret smoker dissolves in his saliva some of the tobacco protein to which he is sensitive.

Treatment.—Gingivitis or periodontoclasia associated with protein hypersensitiveness is treated ideally by the exclusion of the reagen or the offending substance. The management of the acute gingivitis is relatively simple when the nature of the protein is established, but the larger number of periodontoclasia cases had suffered such organic damage that allergic control was helpful but not curative.

In these cases of allergic disease, where the diet could not be controlled or harmful contacts could not be severed, two other procedures were of value. First, the use of mineral oil before meals proved to be a prophylactic measure.⁸ Theoretically, oil prevents the absorption of undigested proteins from the gastrointestinal tract and holds them in suspension for further digestion or elimination. This method has worked out well in some cases of wheat sensitiveness where deprivation of wheat would be an economic impossibility or a nutritional hazard.

The second form of palliative treatment employed with success in those cases demonstrating a toxic urinary proteose was the oral administration of the glyceryl esters of ricinoleic acid.

CASE HISTORIES

Since the tabulations (Table I) were made, another series of 26 cases has been completed. Twenty of these were definitely allergic in nature. Because of their number it is impossible to present case reports of all, but it seems to be of value to note the case histories of six fairly typical patients.

C A, a twenty one year old male, was referred to the clinic for a recurrent severe gingivitis. His first attack occurred four years ago. It consisted of swelling, ulceration and extreme pain in the gingivae. The lesions at that time and in each subsequent attack extended to the buccal surfaces of the lips, cheeks, pillars, and posterior pharyngeal wall in that order. Because of the discomfort, eating was impossible as the disease progressed. After a period of six to eight weeks, and only when the patient had been positively limited to a milk diet, the condition improved. The patient was seen by the authors two days after the onset of his sixth attack. In blood smears from the gingivae 61 per cent of the white cells were found to be eosinophiles. Skin tests were performed and four plus twenty four hour positive reactions to wheat protease, globulin and glutenin were obtained. These three allergens were then applied to a small area of unaffected buccal mucosa and within fifteen minutes a severe reaction was obtained at the site of wheat globulin inoculation.

The patient was instructed to exclude all wheat from his diet. The condition began to subside in two days and was completely cured in five days.

G D, a twenty six year old female, presented herself at the clinic with a similar history. Her gingivitis, however, had been of eight years' duration. Food protein tests were negative. Bacterial studies had been made and a *Staphylococcus aureus* isolated. With this she had been immunized. No improvement in her oral condition had followed, however. Using bacterial proteins we obtained a very strong reaction, both on the skin and oral mucosa, to *Streptococcus viridans*.

TABLE I

	NO EOSINOPHILIA	EOSINOPHILIA	ALLERGIC HISTORY	CANCER OPIS	POSITIVE SKIN TESTS	URINARY PROTEOSE	ROWE'S DIETS	CURES	IMPROVED
Periodontoclasia	12	74	40	34	62	10	12	16	24
Gingivitis	0	27	16	21	21	5	1	22	5

Desensitization was recommended and the patient was referred to her private physician. At the present time, treatment is incomplete and a report on her progress cannot be made.

R S, a three year old male, was referred by his father, a dentist, because of a severe gingivitis of three weeks' duration. The gums became sore, painful, and swollen suddenly. Fever, inability to eat and prostration were constitutional symptoms. Because of the age local discomfort and uncooperativeness, blood smears were not obtained and skin tests could not be done. Questioning, however, revealed the fact that the child had been presented with a puppy the day of onset. Disposal of the dog was followed by the child's prompt cure. Three months later the child was exposed to another dog. The acute gingivitis recurred.

O O'B, a thirty year old female school teacher who was temporarily employed in a silk dress factory as an inspector complained of tenderness and bleeding of the gums, sensitive teeth, and a peculiar taste in the mouth. Roentgenographs of the teeth showed considerable resorption of the alveolar bone. The gums were hypertrophic and bled easily on pressure. There was a gingival eosinophilia of 18 per cent.

Skin tests revealed only one positive reaction: silk protein. The patient then stated that while working as a dress inspector for the past two years she had been in the habit of biting off loose silk threads and had kept them in her mouth for considerable lengths of time.

A B, a fifty four year old woman, was referred to the clinic for pyorrhea. For several years she had experienced gingival pain on eating very cold or hot foods. The gums bled easily and were sensitive to pressure. Roentgenographs demonstrated considerable alveolar resorption but not sufficient to require a full mouth extraction. There were many gingival pockets from which purulent material and debris could be expressed. There were 17 per cent eosinophiles in the gingival blood smear. She was found to be skin positive to the proteins of tomato, apricot, and yeast.

The patient returned to the clinic in one month in a worse condition than when first seen. Investigation revealed the fact that she had been eating bakers' bread. Two months later, after having been on an absolutely yeast-free diet, the gums were tremendously improved, no purulent matter could be expressed, the interdental tabs were firm and attached, and bleeding on ordinary pressure was absent.

E. B. M., a thirty-eight-year-old mother of five children, was referred for extraction of all remaining teeth.

The first and third left lower molars had been removed five years previously. An extreme degree of pyorrhea existed in the entire right upper and lower molar regions, the process being less pronounced as the midline was approached. Gingival blood studies were interesting in that the degree of eosinophilia corresponded to the extent of pyorrhea. It varied from 28 per cent in right upper and lower second molar region to 12 per cent in the right lateral incisor region to 8 per cent in the gingivae opposite the second left bicuspid. Skin tests revealed a hypersensitiveness to lactalbumin. The interesting feature from the point of view of local trauma and local absorption was the fact that this patient did all her chewing on the right side.

COMMENT

In discussing the probable allergic basis of some forms of gingivitis, one first recalls the frequency of acute lesions in the mouth which follow the ingestion of certain foods. Many patients volunteer the information that canker or soreness of the gums invariably follows the eating of some particular food. Most of these occurrences have been attributed to faulty digestion or inadequate elimination and treated with those conditions in view. Actually, such a form of therapy may be rational, for, if the protein is not quickly altered by the intestinal ferments nor rapidly eliminated, the toxic cleavage product of incomplete protein digestion will be assimilated and cause a reaction. On the other hand, since it is well known that mucous membranes of allergic individuals absorb an offending protein more rapidly than the normal, it may pass through the oral mucosa and exert its effects locally and immediately. The incidence of jagged teeth, overhanging fillings or crowns, malocclusions, and the resultant trauma enhance the absorption of proteins through the abraded or irritated surfaces.

In periodontoelasia, the capillary dilatation, increase of tissue fluid, and spasm of the arteries and arterioles account for the primary alteration of the gingivae and the formation of calculus. With continuance of the arterial spasm the vessels become sclerosed, the blood supply is insufficient, and there is atrophy of the tissues which are dependent upon these vessels for their nutrition. The periodontal fibers break down and cannot be replaced because the proper nutrition is lacking. Manipulation of the gums and removal of calculus help by relieving pressure and temporarily improve circulation by the counterirritant effect of the local trauma. Permanent improvement is rare, however, for a vicious cycle has been established.

Ten cases of gingivitis of pregnancy were studied. In six cases, a local gingival eosinophilia was present. All six had several positive skin reactions to proteins and each was allergic to lactalbumin, lactoglobulin, or casein. On such slight evidence, we hesitate to draw conclusions. Nevertheless, when one realizes that milk proteins are very common allergens, he questions the theoretical necessity of large amounts of milk in the gravid woman's prescribed diet.

In summarizing these cases, which we believe to be of an allergic nature, it may be said that they have certain diagnostic features in common: cosino-

philia, capillary dilatation, increased permeability, exudation. Other important findings in several cases were history of food idiosyncrasies or familial predisposition, positive skin reactions, and positive skin tests with urinary proteose

REFERENCES

- 1 Delater, G. Epulis et pyorrhée sont ordinairement des suites évolutives d'une gingivite latent initiale, *Rev de stomatol* 29: 664, 1927
- 2 Daley, Francis H. A Review of a Six Year Study of Vincent's Infection. *J Am Coll Dentists* 1: 56, 1934
- 3 Rackemann, F. M. Clinical Allergy, New York, 1931, The Macmillan Co
- 4 Herli, J. C., Gallison, D. T., and Brudno, J. Gastro Intestinal Allergy Associated With Transient Intraventricular Block, *N Eng J Med* 210: 123, 1934
- 5 Balyent, R. M. Allergic Diseases, Philadelphia, F. A. Davis Co
- 6 Smul, J. S. Gastro Intestinal Diseases and Food Allergy, *M J & Rec* 135: 80, 1932
- 7 Rowe, A. H. Elimination Diets for Diagnosis and Treatment of Food Allergy, *J Allergy* 2: 92, 1931
- 8 Richet, C., Jr., and Butler, V. Le traitement de l'anaphylaxie alimentaire par l'huile de paraffine, *Presse med* 40: 925, 1932

416 HUNTINGTON AVENUE

DEPARTMENT OF CLINICAL MEDICINE

TUFTS COLLEGE DENTAL SCHOOL

BLOOD GROUPING IN THE INFECTIOUS DISEASES*

HENRY BRODY, M.D. LAWRENCE W. SMITH, M.D. AND WILLIAM I. WOLFF
NEW YORK, N. Y.

IT HAS been maintained for some time that there is a constitutional factor in susceptibility to infectious disease. One avenue of approach to the study of this problem has been a statistical investigation of the incidence of the four blood groups in patients with the various infectious diseases.

A number of such studies have been published. In acute anterior poliomyelitis, Grooten and Kossovitch (1930) noted a marked predominance of Group A over Group O, a complete absence of Group AB, and a slight diminution of Group B, among 78 cases in Alsace. Manicattide, Biatescu, and Rusescu (1929), and Marinresco, Manicattide and Diaganescu (1929), agreed upon a predominance of Group A in Roumanian cases. For (1931) reports an appreciable increase in A, and a marked decrease in B, among 22 poliomyelitis patients in Italy. Jungeblut and Smith (1932) report a statistically significant decrease in B and a slight increase in O group, in 125 paralytic cases of poliomyelitis in children over five years of age in New York City. They find a similar decrease in B among 208 polioconvalescent serums. Totalling the findings from the serums and cases, in 551 bloods there are 10.3 per cent B, a decrease from their controls of 3.7 ± 1.7 per cent which is almost of statistical significance. It represents the largest group studied and reported. Shaw, Thelander, and Kilgariff (1932), in 100 cases from California also report a diminution in B and

*From the Laboratory of the Willard Parker Hospital. Department of Hospitals.
Received for publication June 2, 1935.

increase in O, and, strikingly, a complete absence of AB. Hatzky (1933) reports 131 cases in which there is a striking increase in B. With the exception of Hatzky, these various authors agree upon a relative decrease in incidence of individuals belonging to B group in patients with poliomyelitis.

In diphtheria, on the basis of Schick tests, Hirszfeld, Hirszfeld, and Brokman (1924), Nowak (1931), and Farjot (1933) all agree there is no difference in immunity to diphtheria in varying blood groups.

In 36 scarlet fever cases, Mironesco and Stefanov (1926) report a somewhat lowered incidence in Group A and a striking increase in Group B. Nowak (1932), studying 232 cases in Vienna, also finds a slight decrease in Groups A and B. On the other hand, Körwer (1932), in Germany, finds in 363 cases a distribution no different than in his control series, while Kiss and Teveli (1930) find relatively large decreases in both A and B in 172 cases of scarlet fever in Budapest.

In rubella and pertussis, the only report found in the literature is the one of Mironesco and Stefanov (1926), in which are reported 64 cases of rubella, with a slight decrease in Group B, and 14 cases of pertussis, none of which belongs to Group B.

We have collected from the transfusion records of Willard Parker Hospital, a New York City Hospital for contagious diseases, the blood groups of patients typed in the years 1932, 1933, 1934, and 1935. There are 440 individuals represented in this list, 29 of these having had double and, in two instances triple, infections. We are indebted to Dr. K. P. Lorenz, pathologist to the Kingston Avenue Hospital, New York City, for an additional 127 typings. This paper is based upon these 567 patients.

It is obvious that a proper evaluation of the distributions of the blood groups in the several contagious diseases, necessitates a comparison with the "normal" distribution. The considerable variation in incidence of blood groups in different races makes it important to control this factor. This is particularly true in a large metropolitan hospital. Whether or not there has been a stabilization of the racial proportions—and of the distribution of the four blood groups—in a large city such as New York, is not certain. Tiber (1930) has suggested that the lower incidence of Group A in his large series from Bellevue Hospital, as compared with the earlier American reports of Moss (1910), Karsner (1918), and Snyder (1929), is due to the relatively high incidence of South Europeans among the patients at Bellevue Hospital. Similarly low figures for Group A are reported, however, by Culpepper and Ableson (1921) in Detroit, by Seeger and Schaefer (1933) in Milwaukee, and by Jungeblut and Smith (1932) from the Presbyterian Hospital in New York, the correspondence of all of which percentages with those of Tiber is very striking.

For our control group, we have used the distribution obtained from 1,000 individuals, typed as prospective donors for patients at Willard Parker Hospital. They are parents, relatives, and friends of the patients in the hospital (professional donors have been omitted), and therefore presumably reflect the racial distribution of the patients for whom they were typed. The incidence of the various groups differs very slightly from those reported by Tiber, Jungeblut and Smith, Seeger and Schaefer, and Culpepper and Ableson.

In 302 cases of scarlet fever, 45.4 per cent belong to Group O, 30.8 per cent to Group A, 17.9 per cent to Group B, and 6.0 per cent to Group AB. The only differences of any magnitude, as compared with the control group, are seen

TABLE I
NORMAL DISTRIBUTION OF BLOOD GROUPS IN AMERICAN CITIES

		O	A	B	AB
Culpepper and Ableson	Detroit	5,000	44.5%	36.0%	14.3%
Seeger and Schreier	Milwaukee	489	42.1	36.2	13.9
Tiber	Bellevue Hospital, N. Y. C.	10,000	45.6	36.4	13.5
Jungeblut and Smith	Presbyterian Hosp., N. Y. C.	1,000	45.6	35.7	14.0
Authors'	Willard Parker Hosp., N. Y. C.	1,000	47.8	35.3	12.2

in Groups A and B. In Group A, there is a diminution of 4.5 per cent \pm a standard deviation of 3.06 per cent. The decrease in incidence of Group A is of the same order of magnitude as those reported by Nowak who found a decrease of 6.5 ± 4.0 per cent, and Kiss and Tevelh who found a decrease of 9.98 per cent. The figures of Minnesco and Stefanov are too few and too inadequately controlled to be of any significance. The series reported by Korwei show no significant changes in any of the groups from his controls. None of these differences (with the possible exception of Kiss and Tevelh's, who do not give sufficient data to treat statistically) reach statistical significance. In Group O, the authors and Korwei find a slight decrease, while Nowak and Kiss and Tevelh both find a rather large increase. In Group B, Nowak and Kiss and Tevelh find decreases, which is opposite to our increase. This increase of 5.7 ± 2.44 per cent almost attains the rigid statistical significance of probability of 1 in 100 that the difference is not a reflection merely of random sampling (i.e., that there are too few cases to allow the laws of chance distribution to operate), its probability being 0.019. In Group AB, the authors, Nowak, and Korwei all find an insignificant increase, while Kiss and Tevelh find a slight decrease.

In the other infectious diseases studied, pertussis, rubella, varicella, and diphtheria, we find in all a striking decrease in those of Group B. The numbers of cases of varicella and diphtheria are too small to merit discussion. In measles, the incidence of Group B is decreased by 4.5 ± 2.56 per cent, and in pertussis by 10.4 ± 1.64 per cent. The first value has a probability of only 8 in 100 that the difference is due to sampling, and for the second, p becomes so exceedingly small that it may be said with complete assurance that the decrease is statistically significant. This agreement between measles and pertussis is suggestive when the similarity of the underlying pathologic process is remembered (McCordock and Muckenfuss, L. W. Smith). If one combines the results obtained in these two pathologically similar diseases, the decrease in Group B is found to be 7.0 ± 1.79 per cent, the p for which value is 1 in 10,000. If one compares the Chi square for the distribution of the blood groups in these diseases with the control distribution, it is found to be 12.05, well above the critical value of 11.34 which indicates a probability of 1 part in 100.

This decrease in Group B in measles and pertussis becomes of even more interest when it is recalled that in poliomyelitis, a statistically significant de-

TABLE II
STATISTICAL ANALYSIS OF THE DISTRIBUTION OF THE BLOOD GROUPS IN THE CONTAGIOUS DISEASES

DISEASE	No	O				A				B				AB			
		No	%	σ	DIFF FROM CONTROL	p*	No	%	σ	DIFF FROM CONTROL	p*	No	%	σ	DIFF FROM CONTROL	p*	chi square
Controls	1000	478	47.8 ± 1.58	—	—	—	353	35.3 ± 1.51	—	—	—	122	12.2 ± 1.03	—	—	—	—
Scarlet fever	302	137	45.4 ± 2.86	-2.4 ± 1.27	0.47	—	93	30.8 ± 2.66	+5 ± 3.06	0.14	—	54	17.9 ± 2.21	+5.7 ± 2.44	+1.3 ± 1.51	0.36†	8.0
Measles	130	71	54.6 ± 2.27	+6.8 ± 4.53	0.13	—	41	31.6 ± 4.06	-3.7 ± 4.33	0.39	—	10	7.7 ± 2.34	-4.5 ± 2.56	+1.5 ± 2.22	0.49†	4.0†
Pertussis	109	59	54.1 ± 2.77	+6.3 ± 5.03	0.21	—	38	34.9 ± 4.56	-0.4 ± 4.80	0.94	—	2	1.8 ± 1.27	-10.4 ± 1.64	+1.5 ± 2.86	0.117	14.2
Whooping cough	78	16	42.2 ± 8.02	-5.6 ± 8.16	0.49	—	16	22 ± 8.02	+6.9 ± 8.15	0.40	—	4	10.5 ± 4.98	-1.7 ± 5.18	+0.6 ± 3.70	0.87	0.85
Diphtheria	21	12	57.2 ± 10.83	+9.4 ± 10.97	0.39	—	7	33.3 ± 10.28	-2.0 ± 10.30	0.85	—	1	4.8 ± 4.67	-7.4 ± 4.78	+0.1 ± 4.72	0.98†	0.13

*p, the probability that the difference arises merely due to random sampling, is arrived at by dividing the difference by σ , its standard deviation, and then interpolating this quotient in a table of probabilities (Dunn, 1929).

†N², Chi square, a measure of the significance of differences between distributions, is obtained as explained in Dunn's paper, "Statistical Methods in Physiology." Differences between the distributions in the blood groups in the various diseases and the control series are significant when N² becomes greater than 11.34.

**p, for the Chi square values, is obtained by interpolating in a suitable nomogram (Dunn, p. 341).

crease in incidence of Group B was also found. Measles and poliomyelitis are both diseases of virus etiology; pertussis is at least suspected of having a virus as an etiologic agent. It might prove of interest, therefore, to ascertain the blood groups of a large number of individuals with other diseases of virus etiology, or suspected of having a virus etiology, e.g., variola, yellow fever, epidemic parotitis and influenza. Should all of these show a comparatively lower incidence among individuals of Group B, this might serve as a serologic approach to the prophylaxis, and possibly therapy, of these diseases, as has been recently suggested for poliomyelitis by Jungeblut (1933).

SUMMARY

1. There have been 567 individuals with the common infectious diseases typed, and the distribution among the four blood groups compared with a normal distribution.

2. In scarlet fever there is a decrease in incidence of individuals of Group A and an increase in Group B in pertussis, rubella and perhaps varicella, to which may be added poliomyelitis as studied previously by one of us. There is a significantly decreased incidence in Group B. Pertussis furthermore, shows a notable increase among individuals of Group AB.

3. A possible relation of these findings to immunity is suggested.

REFERENCES

- Culpepper, W. L., and Ableson, M. Report on 5,000 Bloods Typed Using Moss's Grouping, *J. Lab. & Clin. Med.* 6: 276, 1921.
- Dunn, H. Application of Statistical Methods in Physiology, *Physiol. Rev.* 9: 275, 1929.
- Farjot, A. Reaction de Schick et groupes sanguins. *Compt. rend. Soc. de biol.* 113: 773, 1933.
- Foa, A. Sopra un nuovo caso di encefalite post-vaccinica, *Pediatr. d. med. prat.* 6: 24, 1931.
- Grooten, O., and Kossovitch, N. Sur les groupes sanguins chez les enfants poliomyelitiques. *Compt. rend. Soc. de biol.* 105: 125, 1920.
- Hatzky, K. Untersuchungen über die Blutgruppenverteilung bei Poliomyelitikern, *München. med. Wchnschr.* 80: 197, 1932.
- Hirszfeld, H., Hirszfeld, L., and Brokman, H. On the Susceptibility to Diphtheria (Schick Test Positive) With Reference to the Inheritance of Blood Groups, *J. Immunol.* 9: 571, 1924.
- Jungeblut, C. W. The Power of Normal Human Sera to Inactivate the Virus of Poliomyelitis in Its Relation to Blood Grouping in Exposure, *J. Immunol.* 24: 157, 1933.
- Jungeblut, C. W., and Smith, L. W. Blood Grouping in Poliomyelitis: Its Relation to Susceptibility and the Neutralizing Property of Convalescing Sera, *J. Immunol.* 23: 35, 1932.
- Karsner, H. T. Transfusion With Tested Bloods. *J. A. M. A.* 70: 769, 1918.
- Kiss, P., and Tevel, Z. Blutgruppe und Scharlach. *Jahrb. f. Kinderh.* 127: 110, 1930.
- Korwar, H. Blutgruppe und Scharlach, *Jahrb. f. Kinderh.* 136: 59, 1932.
- McCordock, H., and Muckenfuss, R. Similarity of Virus Pneumonia in Animals to Epidemic Influenza and Interstitial Broncho-Pneumonia in Man, *Am. J. Path.* 9: 221, 1932.
- Manicattide, M., Bratescu, A., and Rutescu, A. Beobachtungen betreffend die erste Kinder-Jahmungs-Epidemie in Rumänien. *Ztschr. f. Kinderh.* 48: 125, 1920.
- Marinesco, G., Manicattide, M., and Dragulesco, S. Etude clinicothérapeutique et anatomopathologique de la poliomyélite humaine qui a sévi en Roumanie pendant l'année 1927, *Ann. de l'Inst. Pasteur* 43: 223, 1929.
- Mironesco, T., and Stefanov, G. Contribution à l'étude du rapport qui existe entre les groupes sanguins et les infections. *Compt. rend. Soc. de biol.* 95: 140, 1926.
- Moss, W. L. Studies in Isoagglutinins and Isohemolysins, *Bull. Johns Hopkins Hosp.* 21: 63, 1910.
- Nowak, H. Besteht ein Unterschied in der Häufigkeit der verschiedenen Blutgruppen bei den Drüsenorgenen? *Monatsschr. f. Kinderh.* 54: 74, 1932.

- Seeger, S. J., and Schaefer, A. A.: Blood Grouping, *Am. J. Dis. Child.* 45: 999, 1933.
Shaw, E. B., Thelander, H. E., and Kilgariff, K.: Blood Grouping in Poliomyelitis, *J. Pediat.* 1: 346, 1932.
Smith, L. W.: The Pathology of Measles With Special Reference to Pneumonia, *Arch. Path.* 18: 761, 1934.
Snyder, L. H.: Blood Grouping in Relation to Legal and Clinical Medicine, Baltimore, 1929, The Williams & Wilkins Co.
Tiber, A. M.: Observations on Blood Grouping and Blood Transfusions, *Ann. Surg.* 91: 481, 1930.
-

A HITHERTO UNDESCRIBED MICROORGANISM OF THE ALCALIGENES GROUP*

ELIZABETH L. HAZEN, AND MARY MORTILLARO, NEW YORK, N. Y.

A BACTERIOLOGIC study of a hitherto undescribed microorganism recovered twice from the blood stream of a fatal case is recorded, together with a brief description of the case.

A specimen of blood for bacteriologic examination was received from a woman, aged forty-one, who lived in Westchester County, New York. The symptoms reported were: septic temperature (99 to 104° F.), chills, profuse sweating, extreme prostration, and abdominal distention. The erythrocyte count was said to be 4,080,000, and the leucocyte count 13,500. A differential blood count later showed 91 per cent polymorphonuclear neutrophils, 8.7 per cent lymphocytes, and 0.3 per cent large mononuclears. A diagnosis of typhoid fever was first suggested, but later "undulant fever or a bacteremia" was considered. The patient's blood serum during the third and fourth week after the onset of illness did not agglutinate *B. typhosus*, *B. abortus* (bovine), or *B. tularensis*. No microorganism of the enteric disease group was recovered from the feces. The patient died on the forty-sixth day of illness; an autopsy was not performed. Additional information concerning the case revealed that one week prior to her illness she had returned from a visit on a farm in Virginia where raw milk was used. Only a few days before returning home she had dressed a quail and a rabbit which had been killed by a hunter.

BACTERIOLOGIC STUDY

A small gram-negative aerobic bacillus was isolated in pure culture from the blood specimen which was collected one week before death. Coincidentally, a transplant of a microorganism, recovered from the patient's blood eleven days before death, was received for identification. Preliminary tests indicated that the two cultures, designated as Nos. 3348 and 3349, respectively, were probably homologous and belonged to the *B. alcaligenes* group.

Morphology.—The microorganisms were gram-negative rods that varied from coccoid to bacillary forms; frequently a faintly stained transverse central

*From the Division of Laboratories and Research, New York State Department of Health, Branch Laboratory.

Received for publication, July 2, 1935.

brand was observed. They occurred singly or in long or short chains, no flagella, capsules, or polar bodies were demonstrable.

Cultural Characteristics—On plates of meat infusion agar and horse blood hormone agar the colonies were colorless, glistening, opaque, convex, and friable, with entire edges, and on the blood medium there was no hemolysis. In gelatin tubes after forty eight hours at 37° C growth was moderate and flaky. No liquefaction occurred during a three weeks' period of incubation. The growth was moderate and of light cream color on potato slants soaked in a 0.1 per cent sodium carbonate solution, while on the more acid or glycerol potato medium no growth occurred. In meat infusion broth the cultures showed (after forty eight hours) slight turbidity with a faint ring and a granular deposit which did not disintegrate on shaking.

Biochemical Reactions—Litmus milk was unchanged. In milk containing either phenol red or brom cresol purple a slightly alkaline reaction was observed. Indol was not formed. Acetyl methyl chitinol was not demonstrated and there was no color change in the methyl red test. None of the carbohydrates (in peptone water medium) commonly used was fermented, but a strong alkaline reaction was produced in the medium. The microorganisms grew best at 37° C, and indifferently or not at all at 20° C. Exposure to moist heat for five minutes at 60° C resulted in their death.

SEROLOGIC STUDY

Agglutinating serums were produced in rabbits by repeated intraperitoneal injections of the living microorganisms. As difficulty was encountered in obtaining homogeneous suspensions of the microorganisms, the living cultures were ground before making the suspensions for agglutination purposes. Complete reciprocal agglutination was obtained with cultures 3348 and 3349 and their homologous antisera in dilutions of 1:1,200. These cultures also reciprocally absorbed the agglutinative properties from antisera 3348 and 3349 (indicating the identity of the two microorganisms). *B. typhosus*, *B. paratyphosus* A and B, *B. abortus* (bovine), *B. melitensis*, *B. paramelitensis*, *B. whitmorei*, *B. bronchisepticus*, *Actinobacillus lignieresii*, and *B. tularensis* were not agglutinated in the antisera 3348 and 3349, nor were cultures 3348 and 3349 agglutinated in the antisera of the microorganisms just mentioned.

PATHOGENICITY

Rabbits, white rats, and white mice were not found to be susceptible. Guinea pigs injected intraperitoneally with the original cultures (growth from one or two eighteen hour agar slants, suspended in 1 cc of physiologic salt solution) exhibited a variable susceptibility. Some of the guinea pigs became acutely ill and died within twenty four hours to five days, others, showing only malaise or no symptoms, survived indefinitely. The animals that died of an acute infection showed evidence of acute peritonitis. The microorganisms were cultivated from the peritoneal exudate, the liver, spleen, and mesenteric lymph glands. The passage cultures proved to be infectious for guinea pigs upon intraperitoneal injection.

CLASSIFICATION

Before classifying cultures 3348 and 3349, certain of their cultural and biochemical characteristics were compared with those of the microorganisms which they resembled in many respects, and differences from these same microorganisms (members of the abortus melitensis group and *B. bronchisepticus*) were noted.

There was a striking resemblance between the microorganism and *B. bronchisepticus* in growth on solid media and in morphology, but they differed in many important respects. As Wilson¹ reports, *B. bronchisepticus* produces an intense alkalinity in litmus milk, the alkalinity beginning at the surface and progressing downward until finally the medium is of a blue black color. On potato medium the growth is abundant, moist, and glistening, and of a light tan color after twenty-four hours; in two or three days the color is coffee or Van Dyck brown, the potato itself becoming dark brown. Cultures 3348 and 3349 did not produce a detectable color change in litmus milk after three weeks' growth. They grew on potato slants only if the slants had been soaked in sodium carbonate solution. The growth on this medium was moderate after four or five days and was of a creamy color. Also, *B. bronchisepticus* is a motile bacillus, whereas cultures 3348 and 3349 were nonmotile. However, the failure to demonstrate motility in the two microorganisms is not of great significance as it is a well-known fact that variations of this type may occur in many species of motile bacteria.

While cultures 3348 and 3349 resembled members of the abortus melitensis group in certain of their cultural and biochemical reactions, they were immunologically unrelated to this group and did not produce a chronic disease in the guinea pig. Microorganisms of the abortus melitensis group are serologically related and produce chronic lesions, resembling tuberculosis, in the guinea pig.

The *B. alcaligenes* group is generally considered to be composed of gram-negative bacilli that do not ferment carbohydrates and do not form acetyl-methyl-carbinol. This unidentified strain fulfilled these criteria. For convenience of reference to the literature, therefore, it seemed advisable to place the microorganism in this group. However, it should be pointed out that the strain has very few characteristics in common with the type species, *B. fecalis alcaligenes*, which is a motile bacillus with peritrichous flagella, produces an intense alkalization of litmus milk, grows well on ordinary media, and is not sensitive to environmental changes. Cultures 3348 and 3349 are nonmotile, do not possess flagella, produce only a slight alkalization of milk, are fastidious in their media requirements, and are highly sensitive to environmental changes.

SUMMARY AND CONCLUSIONS

The homologous gram-negative bacilli cultivated from two blood specimens obtained from a fatal case of generalized infection were compared with *B. bronchisepticus* and the members of the abortus melitensis group. The significant differences and similarities between this and the heterologous microorganisms were pointed out. Distinctive morphologic, cultural, and biochemical characteristics indicate that it is a representative of a new and hitherto un-

described species. Because of certain characteristics, the microorganism has been placed within the *B. alcaligenes* group, although it differs in many respects from the type species *B. fecalis alcaligenes*.

It is entirely possible that infections due to this pathogen have been overlooked because of the similarity of the clinical symptoms to those of other well known diseases which are characterized by a bacteremia.

REFERENCES

1. Wilson, W. J. The *B. alcaligenes* Group. In: Medical Research Council, A System of Bacteriology in Relation to Medicine. London: His Majesty's Stationery Office, 1929, p. 298.

NORMAL MAGNESIUM METABOLISM AND ITS SIGNIFICANT DISTURBANCES*

BURNHAM S. WALKER, PH.D., M.D. AND ELISABETH W. WALKER, A.M.
BOSTON, MASS.

ALTHOUGH for many years satisfactory methods have been available for the estimation of magnesium in body fluids, such determinations have received little attention from clinical chemists. Our attempt in this paper is to review briefly some of the more important physiologic relationships of magnesium, note certain variations in abnormal states, as well as present a series of magnesium determinations on healthy and diseased individuals. We wish to emphasize in particular the retention of magnesium which occurs in some cases of renal disease, a fact denied by some writers on the subject, and its significance in diagnosis, prognosis and treatment.

Since practically all foods in the human diet contain an adequate amount of magnesium to meet the body's requirements (Joachimoglu and Panopoulos¹), the problem of specific deficiency in magnesium does not arise in human nutrition. The effect of magnesium deprivation in animals, leading to a fall in serum magnesium and the typical magnesium tetany, has been observed and described by the group in D. V. McCollum's laboratory.^{2, 3, 4, 5}

Numerous attempts have been made to associate dietary deficiency in magnesium with increased incidence of malignant neoplasms. This subject is thoroughly reviewed by M. J. Shear⁶ who finds the evidence contradictory and insufficient.

In regard to the effects of excessive magnesium intake, the only study with human subjects which has come to our attention is that of Carswell and Winter,⁷ their experiments lasted twenty and twenty-four days, and consisted of the addition of 8 gm. of magnesium lactate to a uniform diet adequate in phosphorus. They were able to show that about half of the ingested magnesium was absorbed. Calcium balance was positive in both cases, arguing against the

*From the Evans Memorial (Massachusetts Memorial Hospitals) and Boston University School of Medicine, Boston, Mass.

Received for publication July 4, 1953.

the dissolved precipitate, still in the centrifuge tube, were added water to 10 c.c., 1.5 c.c. Molybdate III, and 0.6 c.c. of the reducing agent. After five minutes for color development, the volume was made up to 15 c.c. and the color compared with that of a simultaneously prepared standard made in a 100 c.c. volumetric flask by adding, in order, 5 c.c. standard phosphate solution (containing 0.4 mg. P equivalent to 0.314 mg. Mg), 50 c.c. water, 20 c.c. 2.5 N sulphuric acid, 10 c.c. Molybdate III, 4 c.c. reducing agent, and water to 100 c.c. volume.

These older methods were used from simple personal preference to the newer 8-hydroxyquinoline methods. The results have been shown to be identical (Greenberg and others⁴²). Numerous colorimetric methods based upon the use of dyestuffs have been proposed (Beeka,⁴³ Lang,⁴⁴ Thrun⁴⁵). The last two were tried, but the results were not as consistent as with the magnesium ammonium phosphate precipitation.

Normal Blood Values.—Eighty-seven determinations were made, using blood serum obtained from students in the medical school. The values obtained varied between 1.6 and 3.0 mg. per 100 c.c. serum, with a mean value of 2.2 mg. The values are somewhat lower than those obtained by Greenberg and others⁴² in a similar series, and more in accordance with those observed by Beeher³⁸ and by Bomskov⁴⁶ and those stated by Shohl.⁴⁷ Greenberg's mean value is 2.74, his range 2.0 to 3.6; Beeher's range is 1.8 to 2.3, that of Bomskov 1.7 to 2.6. Shohl sets the limits between one and three mg. per 100 c.c.

This group may be supplemented by the somewhat less authentic figures derived from analyses made on 91 miscellaneous medical and surgical patients, with no apparent disturbances of mineral metabolism and elimination. Of this group the mean value was 2.3 mg. per 100 c.c.; the range was from 1.5 to 2.9 mg. Wacker and Fahrig⁴⁸ in a similar series obtained a mean value of 2.28 mg. with a range of 2.03 to 2.97.

Blood samples in both groups were uniformly taken in the morning before breakfast.

Normal Urine Values.—In the group of medical students an attempt was made to correlate urine output over a definite period with the blood levels. This was previously done by one of us for phosphorus (Walker⁴⁹). For magnesium no correlation could be shown. The values for the one-hour excretion varied from 0.3 to 10.9 mg., with an average of 2.6 mg. per hour.

The twenty-four-hour output of magnesium was also observed in the same group; the maximum twenty-four-hour excretion was 307 mg., the minimum 32.5 mg., the mean value 103 mg.

In the group of hospital cases, supposedly normal from the excretory viewpoint, the values noted in the twenty-four-hour collections were definitely lower. The maximum was 243 mg., the minimum 5 mg., and the mean 86.

Abnormal Values.—Certain cases were observed in the hospital at the time of this study with definitely increased levels of serum magnesium.

Mr. A. C., aged forty-six, was admitted Feb. 11, 1934, in a preuremic condition, with a blood pressure of 210/127. He had a convulsion about two hours after admission, lasting three or four minutes, followed by coma. His history was of albuminuria and hypertension for the previous eight years, severe bilateral temporal headache for five weeks and frequent vomiting for the preceding twelve hours. At the time of admission he was disoriented and

restless. Physical examination revealed fresh and old hemorrhages in both fundi oculi, heavily furrowed swollen tongue, foul breath, heart size and sounds normal. He was treated by phlebotomy, lumbar puncture, and magnesium sulphate by mouth and intravenously. His condition improved two days after admission, and he was discharged much improved on March 17, 1934. Repeated urine examinations consistently demonstrated a tendency to fixation of specific gravity between 1.009 and 1.013. Albumin was present, and the sediment always contained 3 to 10 leucocytes and 2 to 12 blood discs per high power field. Hyaline, granular, cellular, and wax casts were plentiful in all specimens. Blood examination on admission revealed nonprotein nitrogen 80 mg, uric acid 9.5 mg, creatinine 2.7 mg, and sugar 120 mg per 100 cc. The diagnosis was chronic glomerulonephritis with hypertension and uremia. Two determinations of serum magnesium were made in this case, at the time of admission it was 3.92 mg, and three weeks after admission 2.1 mg. This patient had a recurrence of uremia in January, 1935 and died.

Miss S. J., aged fifty six, was admitted Nov. 26, 1933, for diagnostic study with history of hypertension of fifteen years' standing and of migrainous attacks accompanied by partial loss of vision and fortification scotomata. Examination demonstrated an irregular heart with an aortic systolic murmur and a blood pressure of 222/104. Urine examinations were negative except for a very small trace of albumin and a few finely granular casts. Blood counts were within normal limits. Further cardiac examination failed to confirm the arrhythmia but demonstrated a myocardial impurment suggestive of coronary disease. She was not treated in the hospital. Serum magnesium on admission was 2.10 mg and a week later 2.84 mg.

Mr. A. L., aged thirty four was admitted Sept. 9, 1933, with a history of hypertension and nephritis of two and one half years' duration, with increasingly frequent attacks of headache, nausea and vomiting. Admission examination revealed a blood pressure of 204/126 and an apical systolic cardiac murmur. There was evidence of cardiac enlargement down ward, confirmed by x-ray. He remained in the hospital at this time until Dec. 3, 1933, during which period his symptoms were not brought completely under control. Urine examinations demonstrated a fixation of specific gravity between 1.012 and 1.014, small traces of albumin, rare leucocytes, frequent showers of blood discs and occasional hyaline and coarsely granular casts. Blood morphology was normal. Chemical examination of the blood revealed nonprotein nitrogen 57 mg, uric acid 9.1 mg, creatinine 2.5 mg, and glucose 91 mg per 100 cc.

He was readmitted on March 4, 1934 following a minor accident which brought on a recurrence of the severe headache, nausea and vomiting. Following another long hospital stay, he was discharged on May 26 symptomatically improved with a diagnosis of malignant hypertension, hypertensive heart disease, and chronic nephritis. The prognosis was considered very poor. Laboratory findings were comparable to those of the first admission. Therapeutic lumbar puncture was repeated several times and appeared to be the most effective form of treatment. This patient showed a consistently elevated blood magnesium level as shown by the following results: October 20, 2.55 mg, November 6, 2.07 mg, November 17, 2.50 mg, November 20, 2.58 mg, November 27, 2.26 mg, December 1, 2.34 mg, January 22, 3.25 mg, March 5, 4.65 mg.

Mr. F. R., aged twenty six was admitted Oct. 25, 1933 with a history of hypertension, dyspnea, palpitation and precordial pain for a period of nine months. There had been a suspicion of lead poisoning, not confirmed by laboratory examinations. Examination demonstrated a yellow pallor, bleeding gums, faint systolic apical and aortic murmurs and a fine tremor of the fingers. During his hospital stay there was a progressive change for the worse in his blood chemistry and other renal function tests. He was discharged against advice December 9 and died shortly after his return home. Blood pressure at time of discharge was not significantly changed. Urine examinations demonstrated a definite fixation of sp. gr. at 1.010, traces of albumin and occasional casts. He had a marked anemia, 2,000,000 erythrocytes per cmm on admission, 1,700,000 on discharge, with color indices varying between 0.8 and 1.0. Blood chemical examination showed nonprotein nitrogen increasing from 204 to 263 mg, uric acid from 9.1 to 13.2 mg and creatinine from 8.8 to 13.6 mg. His blood sugar was within normal limits. Repeated phenolsulphonephthalein tests gave no

return. The progress of the disease is well illustrated by the increasing values of blood serum magnesium (in mg. per 100 c.c.): October 30, 2.53; November 6, 2.41; November 13, 2.76; November 20, 3.85; November 27, 4.00; December 4, 4.20.

Mr. R. I., aged fifty-two, was admitted Oct. 11, 1933, for study of a hypertensive condition. He also complained of dyspnea, cough with bloody expectoration, edema, and right-sided paresis. Physical findings included glossitis, anterior and posterior cervical adenopathy, râles and dullness in the bases of both lungs, enlarged and tender liver and pitting edema of both legs. The blood pressure was 192/112. A diagnosis of chronic lymphatic leucemia was made within a short time following admission on the basis of the blood examination. The urine was found to contain an enormous number of red blood discs. Chemical examination of the blood gave normal figures for the usual constituents. The phenolsulphonephthalein excretion was 38 per cent in two hours. X-ray gave evidence of considerable effusion in the right pleural cavity. There was no fixation of specific gravity of the urine. Neurologic examination suggested a probable earlier cerebral hemorrhage. He was discharged November 4, unimproved. His serum magnesium was 3.30 mg. per 100 c.c. on admission, later normal values of 2.57 and of 2.66 were obtained. He died soon after his discharge from the hospital.

Mrs. E. T., aged twenty-three, was admitted Feb. 14, 1934, for treatment of pernicious vomiting of pregnancy. Her last period was Jan. 7, 1934, and the vomiting had been of two days' duration and of such severity that she had been unable to retain food or fluids. She was suffering from constant thirst, nausea, severe occipital headaches, and sense of pressure in the lower abdomen. During the last year she had had two pregnancies, both of which had been terminated by curettage on account of the excessive vomiting. Physical examination was noninformative except for signs of dehydration and generalized abdominal tenderness and rigidity. Pregnancy was confirmed by the Aschheim-Zondek test. The vomiting was controlled by use of intravenous glucose together with sedation by phenobarbital. She remained in the hospital until March 7 with only one further episode of severe vomiting, which was eventually controlled by gastric lavage. Urine examination revealed leucocytes in varying and sometimes large numbers. Blood morphology was normal as well as routine blood chemical examination. A single blood magnesium determination done on the day of admission was 3.25 mg. per 100 c.c.

Wacker and Fahrig⁴⁸ in a series of patients with hypertension demonstrated an increase in serum magnesium from their normal mean value of 2.28 to 2.41 mg. This slight difference hardly appears significant, but it is of interest to note that out of our six cases showing increased serum magnesium five had hypertension, the other being a toxemia of pregnancy. Weil, Guillaumin and Weismann-Netter⁵⁰ also have observed increased value of serum magnesium in a few cases of essential hypertension. Their figures deviate so far, however, from the standards used by other authors that comparison is not possible.

In our hospital series there were fifteen other cases of hypertension, none showing any signs of severe kidney damage. Of this group, the maximal magnesium value in blood serum was 2.72, the minimal 1.86, and the mean 2.36 mg. per 100 c.c. This is definitely and probably significantly higher than the means of the normal and of the hospital series (2.2 and 2.3 mg. respectively).

SUMMARY

1. The recent literature on the pathologic chemistry of magnesium has been briefly reviewed.

2. The range of serum magnesium in fasting normal active young adults (87 subjects) is found to be 1.6 to 3.0 mg. per 100 c.c., with a mean value of 2.2 mg.

3 The range in a group of 91 miscellaneous medical and surgical hospital cases with no apparent disturbance of mineral metabolism was 1.5 to 2.9 mg with a mean value of 2.3 mg

4 The twenty four hour urinary output of magnesium in the student group was 32.5 to 307 mg, with a mean value of 103 mg. For the hospital group the maximum was 243 mg, the minimum 5 mg, and the mean 86 mg

5 In five cases of hypertension associated with a greater or lesser degree of renal damage, abnormally high serum magnesium values were found, the highest value being 4.20 mg in a case of terminal nephritis

6 In a group of cases of hypertension without severe renal damage the maximum was 2.72, the minimum 1.86, and the mean 2.36 mg

7 It appears that, contrary to certain statements in the literature, serum magnesium may be elevated in moderate or severe renal insufficiency, especially if associated with hypertension

REFERENCES

- 1 Jochimoglu, and Panopoulos, G. Magnesium Content of Certain Foods, *Med Welt* 3 1538, 1929, in *Chem Abstracts* 25 4065, 1931
- 2 Kruse, H. D., Orent, E. R., and McCollum, E. V. Studies on Magnesium Deficiency in Animals I Symptomatology Resulting From Magnesium Deprivation, *J Biol Chem* 96 519, 1932
- 3 Orent, E. R., Kruse, H. D., and McCollum, E. V. Studies on Magnesium Deficiency in Animals II Species Variation in Symptomatology of Magnesium Deprivation, *Am J Physiol* 101 454, 1932
- 4 Kruse, H. D., Orent, E. R., and McCollum, E. V. Studies on Magnesium Deficiency in Animals III Chemical Changes in the Blood Following Magnesium Deprivation, *J Biol Chem* 100 603, 1933
- 5 Kruse, H. D., Schmidt, M. M., and McCollum, E. V. Studies on Magnesium Deficiency in Animals IV Reaction to Galvanic Stimuli Following Magnesium Deprivation, *Am J Physiol* 105 635, 1933
- 6 Shear, M. J. The Role of Sodium, Potassium, Calcium and Magnesium in Cancer A Review, *Am J Cancer* 18 924, 1933
- 7 Carswell, H. E., and Winter, J. E. The Effect of High and Prolonged Magnesium Lactate Intake Upon the Metabolism of Magnesium and Calcium in Men, *J Biol Chem* 93 411, 1931
- 8 Elmslie, W. P., and Steenbock, H. Calcium and Magnesium Relations in the Animal, *J Biol Chem* 82 611, 1929
- 9 McCarrison, R. Mineral Metabolism and "Stone" Current Sc 1 8, 1932
- 10 Watchorn, E. The Effects of Excessive Intake of Magnesium by the Rat, Especially Concerning the Factors Relating to the Production of Renal Calculi, *J Hyg* 32 156, 1932
- 11 Meyer zu Horste, G. Magnesiumnachts, *Klin Wchnschr* 11 1796, 1932
- 12 Trabucchi, E., and Gazzari, M. Ricerche farmacologiche intorno all'azione del magnesio sul respiro, *Arch di fisiol* 29 536, 1931
- 13 Pinussen, L., and Dimitrijevic, I. N. Über das Verhalten Calcium Magnesium im Blute bei der Narkose, *Klin Wchnschr* 5 849, 1926
- 14 Von Euler, H., Nilsson R., and Auhagen, E. Über die Funktion des Magnesiums beim enzymatischen Kohlenhydratabbau *Ztschr physiol Chem* 200 1 1931
- 15 Lohmann, K. Untersuchungen über die chemische Natur des Kofersments der Milch saurebildung, *Biochem Ztschr* 237 445, 1931
- 16 Jenner, H. D., and Kay, H. D. The Phosphatases of Mammalian Tissues III Magnesium and the Phosphatase System, *J Biol Chem* 93 733, 1931
- 17 Erdtmann, H. Über Nierenphosphatase und ihre Aktivierung, *Ztschr f physiol Chem* 177 211, 1928
- 18 Wacker, L. Zur Kenntnis der Vorgänge bei der Arbeit und Ermüdung des Muskels (Zunahme der Mg und Ca im Blute), *Klin Wchnschr* 8 244, 1929
- 19 Scholtz, H. G. Notiz über die Wirkung des Parathyroiden Hormons auf den Magnesiumgehalt des Blutes, *Arch exper Path Pharmacol* 159 233, 1931
- 20 Greenberg, D. M., and Mackey, M. The Effect of Parathyroid Extract on Blood Magnesium, *J Biol Chem* 98 765, 1932
- 21 Watchorn, E. Serum Magnesium in Relation to Menstruation With a Note on the Calcium, *Brit J Exper Path* 7 120, 1926

22. Cannavo, L.: Hypophysenvorderlappenhormon und Mg-, Ca- und P-gehalt des Blutes, *Biochem. Ztschr.* 245: 234, 1932.
23. Cannavo, L., and Indovina, R.: Einfluss des Prolans auf die Magnesiumbilanz und auf den Magnesiumgehalt verschiedener Organe, *Biochem. Ztschr.* 261: 45, 1933.
24. Howe, P. R.: A New Research on Dental Caries, *Dental Cosmos* 68: 1021, 1926.
25. Kaushansky, L. I.: Chemical Analysis of Teeth, Roots and Crowns Affected by Pyorrhea Alveolaris and Dental Caries. II. Analyses of Phosphorus and Magnesium Content, *Dental Cosmos* 74: 468, 1932.
26. Jacoby, M., and Friedel, H.: Über den Calcium/Magnesium-Quotienten des Blutserums bei der Oxalsäurevergiftung, *Biochem. Ztschr.* 260: 451, 1933.
27. Delbet, P., and Breteau: De l'élimination du magnésium par le bile, *Bull. Acad. de méd. (Paris)* 105: 866, 1931.
28. Delbet, P., and Beauvy, A.: Du magnésium et du calcium dans le bile, *Bull. Acad. de méd. (Paris)* 105: 987, 1931.
29. Erbsen, H., and Damm, E.: Ueber die Kontraktilität der Gallenblase, *Klin. Wehnschr.* 6: 1382, 1927.
30. Bernhardt, A., and Beaver, J.: The Electrodialysis of Human Blood Serum, *J. Biol. Chem.* 69: 113, 1926.
31. Watchorn, E., and McCance, R. A.: Inorganic Constituents of the Cerebrospinal Fluid. II. The Ultrafiltration of Calcium and Magnesium From Human Sera, *Biochem. J.* 26: 54, 1932.
32. Cohen, H.: The Magnesium Content of the Cerebrospinal and Other Body Fluids, *Quart. J. M.* 20: 173, 1927.
33. Stary, Z., Kral, A., and Winternitz, R.: Über die Verteilung der Elektrolyten auf Serum und Liquor cerebrospinalis; Calcium—Magnesium, *Ztschr. f. d. ges. exper. Med.* 66: 671, 1929.
34. Massaut, C.: Le potassium dans le liquide cephalo-rachidien de l'homme et du chien, *Compt. rend. Soc. de biol.* 108: 801, 1931.
35. McCance, R. A., and Watchorn, E.: Inorganic Constituents of Cerebrospinal Fluid. I. Calcium and Magnesium, *Quart. J. M.* 24: 371, 1931.
36. Fishberg, A. M.: Hypertension and Nephritis, ed. 3, Philadelphia, 1934, Lea and Febiger, p. 29.
37. Denis, W., and Hobson, S.: A Study of the Inorganic Constituents of the Blood Serum in Nephritis, *J. Biol. Chem.* 55: 183, 1923.
38. Becher, E.: Der Magnesiumgehalt des Blutes unter normalen und pathologischen Verhältnissen, insbesondere bei Nierenkrankheiten, *Klin. Wehnschr.* 11: 202, 1932.
39. Briggs, A. P.: A Colorimetric Method for the Determination of Small Amounts of Magnesium, *J. Biol. Chem.* 52: 349, 1922.
40. Fiske, C. H., and Subbarow, Y.: The Colorimetric Determination of Phosphorus, *J. Biol. Chem.* 66: 375, 1925.
41. Kramer, B., and Tisdall, F. F.: A Simple Technique for the Determination of Calcium and Magnesium in Small Amounts of Serum, *J. Biol. Chem.* 47: 475, 1921.
42. Greenberg, D. M., Lucia, S. P., Mackey, M. A., and Tufts, E. V.: The Magnesium Content of the Plasma and Red Corpuscles in Human Blood, *J. Biol. Chem.* 100: 139, 1933.
43. Becka, J.: Über die praktische Anwendung der kolorimetrischen Magnesiumbestimmung in der Biochemie, *Biochem. Ztschr.* 233: 118, 1931.
44. Lang, K.: Eine Mikromethode zur Bestimmung kleinster Mengen Magnesium in biologischem Material, *Biochem. Ztschr.* 253: 215, 1932.
45. Thrun, W. E.: Rapid Determination of Small Amounts of Magnesium in the Presence of Phosphate, *Ind. Eng. Chem. Anal. ed.* 4: 426-7, 1932.
46. Bomskov, C.: Determination of Magnesium in Blood With 8-hydroxyquinoline, *J. Biol. Chem.* 99: 17, 1932.
47. Shohl, A. T.: Mineral Metabolism—Calcium and Magnesium, *Ann. Rev. Biochem.* 2: 207, 1933.
48. Wacker, L., and Fahrig, C.: Über die mineralischen und lipoiden Bestandteile des Blutserums bei der essentiellen Hypertension im Vergleich zu den physiologischen Verhältnissen, *Klin. Wehnschr.* 11: 762, 1932.
49. Walker, B. S.: Normal Relationships of Blood and Urine Phosphorus, *J. Lab. & Clin. Med.* 17: 347, 1932.
50. Weil, M., Guillaumin, C., and Weismann-Netter, R.: Le calcium et le magnésium sériques chez l'adulte normal, hypertendu ou athéromateux, *Compt. rend. Soc. de biol.* 88: 732, 1923.

ANTIPNEUMOCOCCUS SERUM CONTAINING HETEROPHILE ANTIBODY*

A LABORATORY AND CLINICAL REPORT

GEORGE E. ROCKWELL, M. A., M. D., AND RICHARD TYLER, M. D.,
CINCINNATI, OHIO

PNEUMONIA in man is common and carries a high mortality. The need of improvement in the treatment of this disease is apparent, and is exemplified by the various studies on the use of antisera, drugs, pneumothorax, and other agents.

About 90 per cent of all cases of this disease are caused by the pneumococcus, the remaining 10 per cent being due to other organisms.¹ The early work of Avery, Chickering, Cole, and Dochez² on acute lobar pneumonia was preceded and followed by many reports on the value and use of various antipneumococcus sera. Recognition of a great multiplicity of types of pneumococcus³ has enhanced the difficulties of the general preparation and use of pneumococcus antiserum. Considered from the type specific aspect, pneumonia, as caused by the pneumococcus, comprises a group of separate diseases, in contrast to such a disease as diphtheria.

The accumulation of reports on the use of antisera in pneumonia has given statistical evidence of the clinical usefulness of antisera of Type I and, to a lesser extent, of Type II. Success in the treatment of pneumonia with antisera is consequently dependent upon early diagnosis, immediate typing of the infecting organism, and prompt administration of the antiserum of the type indicated, with the further practical limitation that the latter must be either Type I or II.

That this procedure is not entirely satisfactory, and that the delay caused by typing the organisms often proves fatal, is evidenced by the fact that Cecil and Suthiff,⁴ and later Park,⁵ have advocated the immediate use of a polyvalent antiserum to be followed later by type specific sera.

Recently our knowledge of the antigenic structure of the pneumococcus has been increased by the discovery of Bailey and Shorb,⁶ that this organism contains heterophile or Forssman antigen.⁷ That the heterophile antibody plays a significant rôle in the defensive mechanisms of the body to pneumonia is suggested by the work of Bailey and Shorb,⁸ and by the report of Powell, Jamieson, Bailey, and Hyde⁹ on the treatment of experimental pneumococcus infection in rabbits with various heterophile antibody antisera. These authors noted that considerable active and passive antipneumococcal immunity is exhibited by heterophile antibody, and subsequently Bailey and Shorb⁹ have reported an increase in the phagocytosis of pneumococci in the presence of combined type specific and heterophile antibodies.

*From the Departments of Bacteriology and Medicine, University of Cincinnati.
Received for publication July 11, 1935.

Since pneumococcus heterophile antigen is species-broad, instead of type specific in distribution, it is evident that such an antiserum, if valuable at all, would be equally useful for all types of pneumococcic pneumonia and would not necessitate typing of the infecting organism. In the past, pneumococcus antiserum that has been used has been mainly of horse origin and hence has contained no heterophile antibody. Therefore, it seemed desirable, in order to throw light on the clinical value of heterophile antisera in pneumococcus pneumonia, to obtain heterophile antibody in quantity, add some of this to the conventional mouse protective horse antipneumococcus serum, and treat available cases of lobar pneumonia with (a) plain heterophile antiserum, and (b) with a mixed antibody serum.

PREPARATION OF HETEROPHILE ANTISERUM AND ANTIPNEUMOCOCCUS SERUM
CONTAINING HETEROPHILE ANTIBODY*

Theoretically, heterophile antigen from any source should be moderately satisfactory for inciting the formation of heterophile antibody for use in antipneumococcus serum. That heterophile antigen of pneumococcus origin should be preferable is indicated by the work of Landsteiner and Levine,¹⁰ and the reports of Bailey⁹ and his associates on absorption reactions of various heterophile antigens and antibodies.

Various animals of the "rabbit type" should suffice for the preparation of ample heterophile antibody. From a practical point of view one is limited to the cow and rabbit as animals most suitable for this purpose, since other domesticated animals such as the horse, sheep, and goat are unsuitable, in that their tissues contain normally a large amount of heterophile antigen, and therefore they do not produce heterophile antibody (*i.e.*, these are of the "guinea pig type").

The first attempts to produce suitable heterophile antibody in large quantity were made by using both calves and rabbits in parallel experiments. After six months of immunization, the experiments with the calves were discontinued because of the uniformly low heterophile titers obtained.

Rabbits proved satisfactory for producing antipneumococcus heterophile antibodies. The limitation in rabbit immunization is the small yield of serum per rabbit and the tedious process of immunization of many animals.

However, we have immunized large groups of rabbits, using from twenty-five to one hundred animals at a time. The animals were inoculated with a suspension of heat-killed pneumococci of about 50 per cent of a mixture of equal parts of Type I, II, III, and Cooper's Type IV, plus approximately 50 per cent of a rough strain known as D.R.I., which is very high in heterophile antigen.⁶

A course of immunization of three to four weeks led to the production of heterophile antibody titers varying from 2,500 units to 25,000 or more units per cubic centimeter, and the production of practically no mouse protective anti-

*We are indebted to H. M. Powell of the Lilly Research Laboratory, Indianapolis, for the description of the preparation and for all the antisera used in the work reported in this paper.

body for any of the types. The mouse tests of course evaluate the type specific Felton antibody, but do not evaluate the species broad heterophile antibody.

Prior to combining it with horse antipneumococcus serum, we have concentrated our rabbit heterophile antipneumococcus serum by salting out on half saturation with ammonium sulphate. The whole globulin precipitate is filtered off from the albumin solution, the latter discarded, and the precipitate dialyzed until it is salt free. The resultant heavy precipitate of euglobulin is removed by centrifugation, the solution is made isotonic to normal serum and filtered through a Berkefeld candle. Methylolate is finally added as a preservative, in a concentration of 1:10,000. Final sterility tests, safety tests, and heterophile antibody potency tests are then conducted in the regular way.

In the preparation of the usual mouse protective antibody, horses have been used. We have utilized for injection Types I, II, III, and Cooper's Type IV pneumococci, of the regular high degree of virulence for mice as maintained by weekly mouse passage. The culture emulsions have been heat killed, and the horse bleedings on attaining sufficient potency have been concentrated and prepared in the usual way for therapeutic use. Mouse protective tests have showed an increasing potency against Types I and II of from four hundred to five hundred units up to one thousand or twelve hundred units, with a gradual improvement in the serum during the period of its use. Although this serum shows a varying mouse protective potency against certain laboratory strains of Type III pneumococcus, this is discounted by the fact that it shows practically no unitage against our heavily encapsulated Type III laboratory strain known as Park III. So called Type III antisera prepared elsewhere have given these same effects in our hands, and little can be expected directly of any weak Type III antibody present, especially in view of previous unsatisfactory clinical experience with Type III mouse protective serum.¹¹ Such "weak" Type III antibody is greatly increased in opsonic action, however, by the addition of heterophile antibody,¹² and such a combination of antibodies is theoretically deserving of a clinical trial. Little can be said at this time about the single Type IV qualities of this serum.

A combined serum was obtained by mixing together heterophile antipneumococcus serum and mouse protective antipneumococcus serum. Generally one-fourth to one-tenth of the mixed serum has comprised heterophile antibody while three-fourths to nine-tenths of the volume has comprised mouse protective antibody. The mixing of the two antisera has been done in a fractional way and the resultant precipitate of heterophile antigen antibody complex (the heterophile antigen coming from the horse serum) has been removed by filtration, before addition of more heterophile antibody.

The various lots or batches of combined heterophile and mouse protective antipneumococcus serum prepared for clinical use as reported below have varied from original potencies of 500 heterophile units and 400 mouse protective Type I and Type II units per cubic centimeter, up to 10,000 heterophile units and 1,000 mouse protective Type I and Type II units per cubic centimeter. It was therefore an expectation that clinical results would be better as the work progressed, all other factors being approximately equal.

CLINICAL USE AND RESULTS

The patients treated with the above described antisera were cases at the Cincinnati General Hospital. Their ages varied from sixteen years to seventy. During the period of time that we were treating these patients, *every case of pneumococcic pneumonia was treated and is included in our figures with the exception of a very few which were not treated solely because of an occasional shortage of antisera. We did not select our cases; those of old as well as of recent onset were treated; and we did not exclude from our figures those patients who died within twenty-four hours after their admission to the hospital. It*

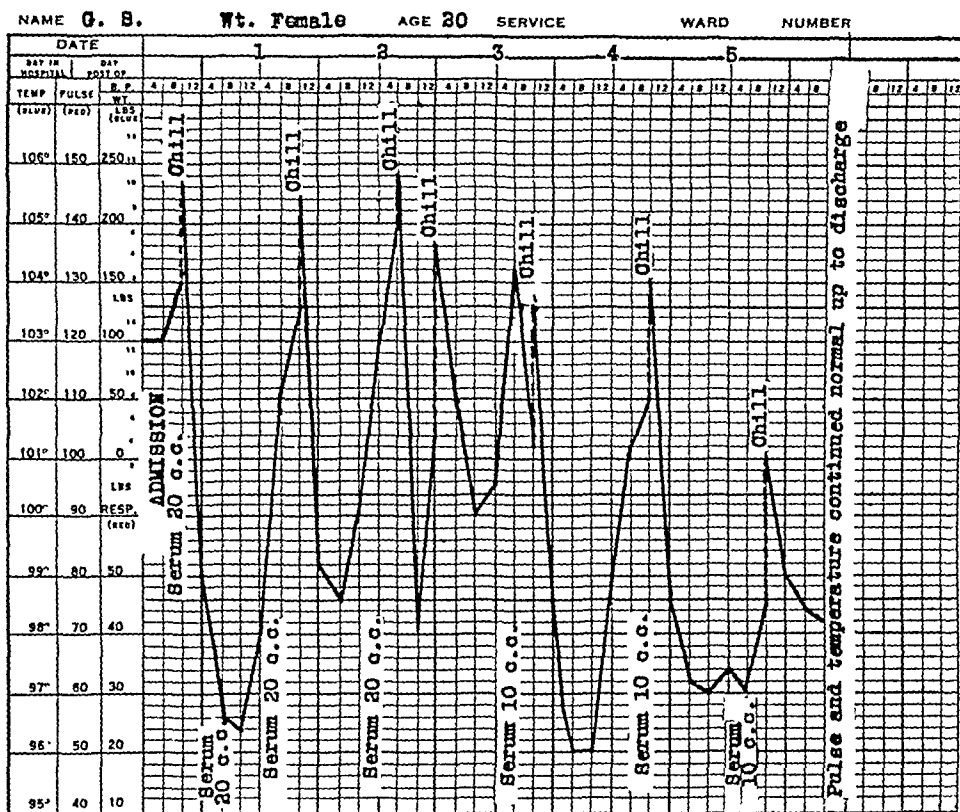


Chart 1.

must be admitted that human pneumonia is such a variable disease from case to case and time to time that only approximate results could be obtained in a statistical way, but spectacularly good results with the heterophile antiserum treatment of case after case would be strong presumptive indication of effectiveness. We are reporting these cases, not for the purpose of giving definite conclusions, but merely to give our own results, on account of the importance of this subject.

When the patient was admitted to the hospital, if a diagnosis of lobar pneumonia was made, an x-ray picture of the chest was obtained, a sample of the sputum was collected for culture and typing by the mouse method, and the

antiserum was given immediately. The patients were tested for sensitiveness to the serum before any of it was given. Only one case was found sensitive, and this individual was not treated, or included in our data. All of the serum was given intravenously. The amount of serum varied, the initial dose being from 10 to 60 c.c. of the concentrate. The serum was given once or twice daily. The total amount of serum given to one patient varied from 40 to as high as 580 c.c. The typical response was a rise in fever with an occasional chill, followed by a rapid fall in temperature to normal or even subnormal. Two such temperature curves are shown in Charts 1 and 2. Not all of the cases responded in this way.

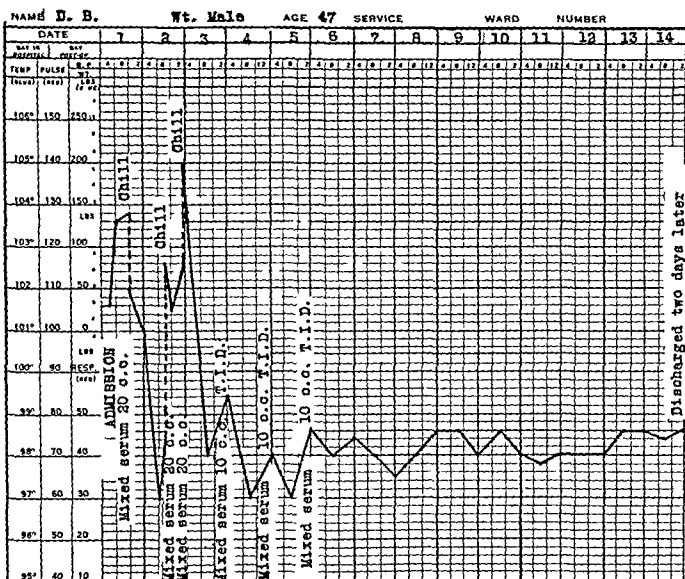


Chart 2

In order to see if normal rabbit serum concentrate would produce a similar reaction it was given to three pneumonia patients. There was no noticeable reaction. They did not have a temperature rise or chill. One of these patients

TABLE I
SHOWING RESULTS OF PNEUMONIA CASES TREATED WITH HETEROPHILE ANTISERUMS
(JANUARY TO APRIL, 1933)

	NO	DIED	RECOVERED	MORTALITY PERCENTAGE
Total	34	16	18	47.0
White	16	6	10	37.5
Black	18	10	8	55.5

was given heterophile antiserum after two injections of normal serum concentrate had failed to produce any reaction or chill. After the heterophile antiserum he had an increase in fever and a chill. This is shown in Charts 3 and 4.

From Jan. 10, 1933 to April 1, 1933 thirty-four patients were treated. The results are shown in Table I.

From Table I, it will be noted that the mortality among the colored patients was higher than for the white patients. Among the sixteen patients who died,

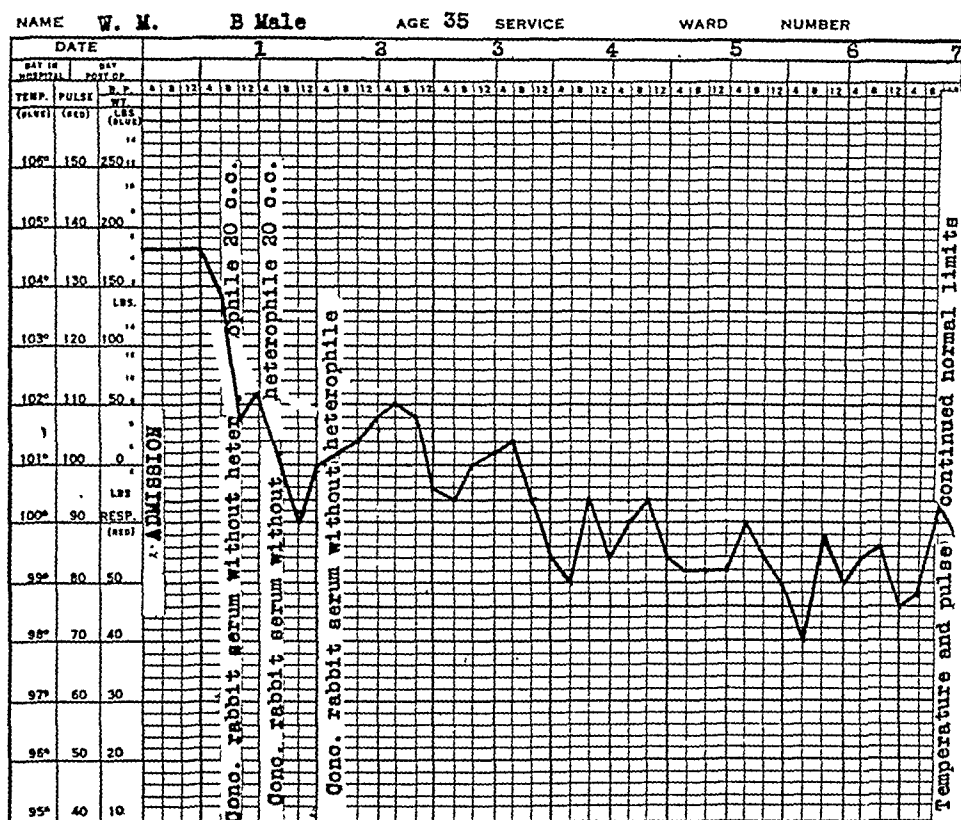


Chart 3.

four did not receive their first antiserum until more than ninety hours after the onset of the disease, one died eighteen hours after admission, and one was a chronic alcoholic.

TABLE II

SHOWING CASES TREATED WITH PLAIN AND COMBINED HETEROPHILE SERUMS
(JANUARY TO APRIL, 1933)

		NO.	DIED	RECOVERED	PERCENTAGE MORTALITY
Treated with plain serum	Total	16	8	8	50.0
	White	8	3	5	37.5
	Black	8	5	3	62.5
Treated with combined serum	Total	18	8	10	44.4
	White	8	3	5	37.5
	Black	10	5	5	50.0

Sixteen of these thirty four patients were treated with plain heterophile serum, while eighteen were treated with mixed heterophile and type specific serum. The separate results are shown in Table II.

The results in Table II suggest that the combined serums might be slightly more effective.

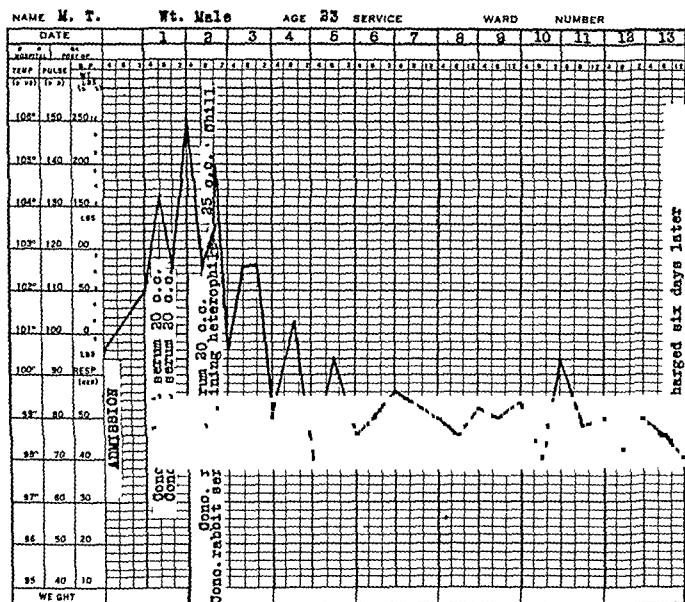


Chart 4

From Oct 25, 1933 to Apr 13, 1934 we treated thirty four additional patients. The results with these cases are shown in Table III.

If we consider all of the 68 cases together the results are shown in Table IV.

TABLE III
SHOWING RESULTS OF PNEUMONIA CASES TREATED WITH COMBINED HETEROPHILE ANTISERUM
(OCTOBER, 1933 TO APRIL 1934)

	NO	DIED	RECOVERED	MORTALITY PERCENTAGE
Total	34	13	21	38.2
White	22	4	18	18.1
Black	12	9	3	75.0

NOTE: Of the white deaths one received serum 72 hours after onset, and the other three patients received their first serum 120 hours after onset. Of the 9 colored patients who died, 4 received their first serum over ninety hours after onset, 4 other patients died less than twenty four hours after admission.

TABLE IV

SHOWING THE TOTAL RESULTS OF ALL OF THE PNEUMONIA CASES TREATED WITH HETEROPHILE ANTISERUMS (JANUARY, 1933, TO APRIL, 1934)

	NO.	DIED	RECOVERED	MORTALITY PERCENTAGE
Total	68	29	39	42.6
White	38	10	28	26.3
Black	30	19	11	63.3

From Table IV it is seen that 29 out of 68 patients died. But 11 of these patients did not receive their first antiserum until more than ninety hours had elapsed after the onset of the disease; 5 other patients died within twenty-four hours of their admission to the hospital, and 1 was a chronic alcoholic.

The heterophile antibody being theoretically effective on all four types of pneumococci, the clinical results of its uses on Types III and IV are interesting and are shown in Table V.

TABLE V

SHOWING THE RESULTS OF TREATMENT OF TYPES III AND IV WITH HETEROPHILE ANTISERUM

	NO.	DIED	RECOVERED	MORTALITY PERCENTAGE
Type III	10	4	6	40.0
Type IV	23	9	14	39.1

SUMMARY

We have given:

1. A brief discussion of the biologic importance of heterophile antibodies to pneumococcal pneumonia in man.
2. A description of the preparation of a pneumococcus antiserum containing heterophile antibodies.
3. The results of the treatment of sixty-eight patients having pneumococcal pneumonia with antiserum containing heterophile antibodies.

REFERENCES

1. Zinsser and Bayne-Jones: Textbook of Bacteriology, ed. 7, New York, 1934, D. Appleton-Century Co., p. 370.
2. Avery, O. T., Chickering, H. T., Cole, Rufus, and Dochez, A. R.: Acute Lobar Pneumonia, monograph VII, Rockefeller Inst. of Med. Res.
3. Cooper, Georgia, Edwards, Marguerite, and Rosenstein, Carolyn: The Separation of Types Among the Pneumococci Hitherto Called Group IV, and the Development of Therapeutic Antiserum for These Types, *J. Exper. Med.* 19: 461, 1929.
4. Cecil, R. L., and Sutliff, W. D.: The Treatment of Lobar Pneumonia With Concentrated Antipneumococcus Serum, *J. A. M. A.* 91: 2035, 1928.
5. Park, W. H., and Cooper G.: Antipneumococcus Serum in Lobar Pneumonia; Administration and Dosage, *J. A. M. A.* 90: 1354, 1929.
6. Bailey, G. H., and Shorb, M. S.: Heterophile Antigen in Pneumococci, *Am. J. Hyg.* 13: 831, 1931.
7. For a review of heterophile antigen and antibody see "The Newer Knowledge of Bacteriology and Immunology," The University of Chicago Press, 1928.
8. Powell, H. M., Jamieson, W. A., Bailey, G. H., and Hyde, R. R.: A Comparative Study of Antipneumococcus Serum Containing Heterophile Antibody, *Am. J. Hyg.* 17: 102, 1933.
9. Bailey, G. H., and Shorb, M. S.: Immunological Relationships of Pneumococci and Other Heterophile Antigens and Biological Significance in Pneumococcus Infections, *Am. J. Hyg.* 17: 358, 1933.
10. Landsteiner, K., and Levine, P.: On the Forssman Antigens in *B. paratyphosus* B and B. dysenteriae Shiga, *J. Immunol.* 22: 75, 1932.
11. Personal communication from Dr. F. D. Felton.
12. Personal communication from Dr. G. Howard Bailey.

ON SOME PRACTICAL CONSEQUENCES OF THE INFLUENCE OF TEMPERATURE UPON ISO AGGLUTINATION*

ISABELLE M. TOWNSEND A.B. AND ARTHUR F. COCA M.D. NEW YORK, N. Y.

THERE are in the literature a few (approximately six) reports of the transfusion of incompatible blood in the usual quantity without a fatal outcome, and in some instances with hardly any reaction whatever. Two of these cases have been studied by staff members of the Donor Bureau of the Blood Transfusion Betterment Association, and in both instances the absence of symptoms was explained by the absence of agglutination of the donor's incompatible blood by the recipient's serum at body temperature.^{1, 2} The question arises as to how often this might occur in similar circumstances.

Moreover, the observed lesser iso agglutination at body temperature suggests that high titered Group O donor bloods which on a quantitative basis according to tests carried out at room temperature have been deemed dangerous for other groups, might be shown to be less dangerous if the tests were conducted at body temperature.

Actually, we have knowledge of one instance, observed in the early history of the Donor Bureau, in which a Group O donor, whose blood, on account of the unusually high titer of anti A iso agglutinins, had been designated as dangerous for transfusion into a Group A recipient, had given 500 cc of blood to a patient who later was identified by test as of Group A. Not the slightest reaction resulted from the transfusion.

We obtained blood from this patient and by the quantitative compatibility test (done at room temperature) we confirmed the impression that the transfused Group O serum should have caused a marked agglutination of the entire blood of the patient.

The study of these questions was taken up by determining the iso agglutinating power of a number of Group A and Group B serums at room temperature with the usual method of serial dilutions, at the same time carrying out a single test at body temperature (about 40° C.) in a proportion of serum and cells with which a rough quantitative comparison could be made at the two temperatures and with which also it could be seen whether at the body temperature the agglutination was absent or very slight. Table I represents an example of such a test.

The cells were used always in a 1:50 suspension, in a quantity of 4 capillary drops. The same capillary pipette was used for distributing the serum dilutions and the cell suspensions. The use of the capillary pipette for this

*From the Donor Bureau of the Blood Transfusion Betterment Association of New York City.

Received for publication July 12 1935

purpose was important as a time saver especially in making the mixtures of the warmed solutions. In the instance cited in Table I the undiluted serum caused only a very slight agglutination at 40°, but a distinctly stronger agglutination with 1 drop of a 1-8 dilution at 17°. It could be estimated from this result that the agglutination was at least 32 times as strong at 17° C. as it was at 40°. The control mixture at 40° was important in aiding the detection of the slight degrees of agglutination and also in a few instances the absence of any agglutination at 40° C.

TABLE I

WARM BATH 40°				COLD BATH 17°							CON- TROL
		CONTROL T.									
Serum, undiluted		4 drops	4 drops	4 drops	2 drops	1 drop					4 drops
Serum, diluted	1- 2					1 drop					
	1- 4						1 drop				
	1- 8							1 drop			
	1-16								1 drop		
Incompatible cells	1-50	4 drops		4 drops	4 drops	4 drops	4 drops	4 drops	4 drops	1 drop	4 drops
Compatible cells	1-50		4 drops								4 drops
Agglutination		±	None	4+	3+	3+	2+	2+	1+	None	None

In order to maintain the "body temperature" mixture at temperatures not lower than 37° while the results were being read, all the tubes containing these mixtures were placed inside a somewhat larger tube containing 1 c.c. of warm water. This column of water filled roughly one-third to one-fourth of the space between the two tubes.

The mixtures for the tests at 40° were made after the serum and cells had been brought to that temperature, having stood in the warm bath for one hour. The capillary pipette used for distributing the cells was first cautiously warmed over a flame and the cell suspension was quickly added to the serums. All mixtures were shaken and allowed to stand in their respective baths for two hours. In those instances in which the clumping (with the undiluted serums) at 40° was 4-plus such serums were also tested at 40° C. in serial dilutions, to determine the approximate and relative agglutinating power.

The results were read with the naked eye, the mixtures being observed for this purpose in the tubes.

Forty-four Group A (anti-B serums) and 9 Group B (anti-A serums) were tested, 53 specimens in all. In Table II are shown the degrees of agglutination of the corresponding agglutinable cells by the 53 undiluted serums at 40° and 17° C.

It is seen that 3 of the serums caused no detectable clumping at 40° C. although they caused a 3-plus or 4-plus agglutination at 17° C. Two other serums caused only the slightest degree of agglutination at 40°, but 2-plus or

4 plus agglutination at 17° C From these results it would seem that nearly 10 per cent of the serums exhibited so little agglutinating power as to make it seem likely that the transfusion of incompatible blood in the respective individuals might have been followed by slight symptoms or none at all By making a rough comparison of the degree of clumping at the two temperatures, an estimate of the relative strength of the agglutination could be made as set forth in Table III

TABLE II

DIFFERENCES IN THE AGGLUTINATION BY THE UNDILUTED SERUM AT 40° C AND 17° C

NUMBER OF SPECIMENS	AGGLUTINATION IN WARM BATH	AGGLUTINATION IN COLD BATH
2	None	3+
1	None	4+
1	±	2+
1	±	4+
3	1+	3+
5	1+	4+
5	2+	3+
17	2+	4+
16	3+	4+
2	4+	4+

TABLE III

NUMBER OF SPECIMENS	6	8	1	16	10	11	1
times stronger in cold bath than in warm bath	64	32	24	16	8	4	2

The summary of these results in Table III shows that in every case the agglutination was stronger at room temperature than at body temperature This relation has had frequent mention by other authors

The serums of 24 "dangerous" Group O (universal) donors were studied in the manner described above The criterion of dangerousness of such donors has been that one part of the donor's serum will produce distinct agglutination of 5 parts of A or B cells, respectively These tests have been carried out at room temperature

When the 24 serums were tested at 40° C in serial dilutions, only 11 were found capable of producing distinct agglutination of 5 parts by volume of the packed cells of the respective incompatible blood These 24 donors were derived from a group of about 350 Group O donors If on a mathematical basis we accept as actually dangerous the 11 whose serums cause distinct agglutination of 5 parts of packed incompatible cells at 40° C, this would give a percentage of approximately 3 When it is considered that other tissues besides the blood elements are capable of taking up the transfused iso agglutinin of the dangerous universal donor, it seems probable that transfusions could be given by at least some of these 11 into Group A or Group B patients without causing symptoms

The result of this study seems to justify the carrying out of the quantitative serum titration for the detection of the so called dangerous universal donor at the body temperature

SUMMARY

1. About 10 per cent of undiluted Group A and Group B serums cause only slight clumping of the corresponding agglutinable cells, or apparently none at all, at body temperature (40° C.). Transfusion of incompatible blood into such patients might be expected to be followed by only mild symptoms or none at all.

2. The quantitative tests used in the detection of the "dangerous" universal donor may be properly carried out at body temperature, the incidence of the "dangerous" universal donor being estimated by this method at approximately 3 per cent of Group O individuals.

REFERENCES

1. Grove, E. F., and Crum, M. J.: Instance of Transfusion of Incompatible Blood Without Reaction, and Source of Error Due to Contamination of Grouping Sera With Mustard Bacillus, *J. LAB. & CLIN. MED.* 16: 259, 1930.
2. Burnham, L.: Transfusion From Group II (A) Donor to Group III (B) Recipient Without Fatal Result, *Arch. Int. Med.* 46: 502, 1930.

THE OXYGEN ABSORBING POWER IN THE PRESENCE OF CERTAIN DISEASES*

D. ROY McCULLAGH, PH.D., T. JAGLENSKI, AND F. KLOBUCAR, CLEVELAND, OHIO

THE amount of oxygen absorbed for each unit of oxygen respired can readily be calculated from a basal metabolic rate chart as has been shown by Ziegler.¹ The percentage of respired oxygen utilized was termed by Ziegler as the "oxygen absorbing power."

The amount of oxygen respired by an individual is calculated by multiplying the respiratory rate by the respiratory amplitude. The amount of oxygen consumed divided by the amount of oxygen respired gives the absorption-respiration ratio, or the oxygen absorption power as given by the following formula:

$$\text{Oxygen absorption power} = \frac{\text{O}_2 \text{ consumption per minute (uncorrected)}}{\text{O}_2 \text{ respired per minute (uncorrected)}} \times 100$$

We studied a group of 366 cases which was unlike Ziegler's in that both males and females of greatly varied ages and condition were included, whereas his study of 160 cases was restricted entirely to relatively healthy males. His first group of cases consisted of young men from the United States Citizen's Conservation Corps who entered the hospital only for the treatment of major or minor surgical conditions. His second group consisted of veterans of the World War. These had various major or minor chronic diseases for the most part, but the majority were convalescing from disabilities received in the war.

Ziegler found that the average oxygen absorbing power was 4.0 per cent, varying from 3.9 per cent in individuals under thirty years of age to 4.1 per

*From the Cleveland Clinic.

Received for publication, July 26, 1935.

cent in persons in the age group above thirty. This age difference is probably insignificant. His point of view is well summarized in the following paragraph:

"In the field of applied physiology, it appears that the oxygen absorbing power will be of great value. The higher the oxygen absorbing power the greater the ability to absorb oxygen from the available supply. The athlete with the highest index would have the best 'wind.' The diver with the higher index would be able to 'stay under' longer. The aviator with the highest oxygen absorbing power could probably go to higher altitudes, and the same would be true for the mountain climber."

We have investigated the oxygen absorbing power of a number of patients who suffered from various disorders, in order to determine whether this measurement under basal conditions is indicative of the respiratory efficiency of the same individual under other conditions.

Since most of the basal metabolic rate studies in our records were made on patients in whom disease of the thyroid gland was suspected, we first wished to determine whether the oxygen absorbing power varied with the amount of oxygen being utilized. We therefore calculated the oxygen absorbing power in a group of 80 patients with basal metabolic rates which varied from minus 30 per cent to plus 81.1 per cent. None of these patients suffered from pulmonary or cardiovascular disorders, nor were any of them known to have anemia.

The total average oxygen absorbing power of patients with basal metabolic rates below normal was $4.23 \text{ S. D. } \pm 0.106$, and for those above normal it was $4.48 \text{ S. D. } \pm 0.093$. The average oxygen absorbing power of patients in both these groups above thirty years of age was 4.36 per cent, and for those below this age limit 4.40 per cent. These figures are close to the average values found by Ziegler. From these findings, we concluded that the oxygen absorbing power under basal conditions was not influenced significantly by age, sex, or metabolic rate.

In studying these two groups, it was interesting to note that certain weak, middle-aged individuals apparently had an oxygen absorbing power equal to that of young, robust patients. This fact would lead one to believe that the oxygen absorbing power under basal conditions is not a measure of the respiratory efficiency during activity.

One might expect that the oxygen absorbing power would be a function of the amount of hemoglobin in the blood. We therefore investigated a group of 50 individuals who were suffering from anemia, and as shown on Table I, the oxygen absorbing power of this group of patients was found to be high. These patients were those who, under normal conditions of activity, were obviously low in respiratory efficiency. It therefore seems that the oxygen absorbing power under basal conditions is not a measure of the respiratory efficiency during activity. The explanation of this probably lies in the fact that in anemia, compensatory mechanisms come into play which permit of a certain amount of activity, but result in overcompensation when the patient is at rest.

Dautrebande² has shown that the cardiac output in anemia is definitely increased. His results show that when the hemoglobin percentage is decreased

from 100 to 50 per cent, the output of the heart is practically normal, but when the hemoglobin percentage is lower than 50 per cent, the output of the heart increases very rapidly.

TABLE I
OXYGEN ABSORPTION POWER IN THE PRESENCE OF CERTAIN DISEASES

	HIGH BASAL METABOLIC RATE		LOW BASAL METABOLIC RATE		ANEMIA	CARDIOVASCULAR DISEASES	PULMONARY DISEASES
	Men	Women	Men	Women			
Number of observations	16	34	10	20	50	51	49
Highest value	5.60	5.50	5.57	5.11	9.18	10.06	4.72
Lowest value	3.55	2.61	3.38	3.17	3.84	3.42	2.26
Average	4.55	4.40	4.39	4.20	5.23	5.15	3.64
Standard deviation from the mean	0.15	0.117	0.178	0.142	0.153	0.154	0.0707
Standard deviation of a single observation	0.189	0.215	0.1784	0.2005	1.323	1.08	0.4960

Hurtado, Kaltrieder, and McCann,³ in their experiments on respiratory adaptation in anoxemia, found that the respiratory response to anoxemia included structural changes in the lungs which consisted chiefly of a widening in the capillaries and a dilatation of the alveoli. These changes were accompanied by a moderate increase of the residual air, and a corresponding decrease in the vital capacity, with little or no change in the pulmonary capacity. There appears to be sufficient evidence to suggest that these changes are compensatory in character. They tend to produce an increase in the surface for diffusion between the circulating blood and the alveolar air, a condition favorable for a more efficient exchange of the respiratory gases, and therefore a higher oxygen absorbing power.

Liljestrand and Stenström⁴ found that in individuals suffering from anemia, the proportion of oxygen in the blood that is utilized by the tissues during rest is greater than in normals. This enables the patient with anemia to maintain the combustion processes during rest with only a moderate increase in the blood flow and the work of the heart.

In a few of the patients whom we examined, the compensatory changes had not been sufficient to make the oxygen absorbing power normal even under resting conditions.

An examination of a series of 51 patients who suffered from cardiovascular lesions showed that the compensatory respiratory changes which occur in diseases of the heart are usually such that the oxygen absorbing power of the patient at rest is greater than that of normal individuals (Table I).

In a group of 49 patients who were suffering from pulmonary disorders, the average oxygen absorbing power was found to be below normal, and the findings (Table I) in this group of patients are in definite contrast to those in the group suffering from cardiovascular diseases or from anemia. The explanation for this is that there appears to be no compensatory mechanism which enables this type of individual to absorb the normal percentage of oxygen inhaled while at rest.

This test obviously is not a measure of the respiratory fitness of individuals for athletic and other endeavors. If the selection depended on these tests, individuals might be chosen who are suffering from a grave cardiovascular disorder or anemia.

The question arose as to whether or not the routine calculation of the oxygen absorbing power of all individuals on whom basal metabolic rates were measured would be of value in discovering undiagnosed cases of anemia or cardiovascular diseases. In this study, 106 patients who were selected at random were investigated. Of this group, thirteen had an oxygen absorbing power over 55 per cent. These individuals were found to have either a cardiovascular disorder or anemia. In each case, however, the clinician in charge had been aware of the condition before the basal metabolic rate determination was made. We therefore concluded that the routine calculation of this index was of little value.

SUMMARY

A study of the oxygen absorbing power of patients under basal conditions shows that it is not a measure of the respiratory efficiency of these individuals while they are active.

The oxygen absorbing power during rest is influenced but slightly, if at all, by changes in thyroid activity.

The oxygen absorbing power usually is normal or above normal in patients suffering from anemia or cardiovascular disorders. In case of patients suffering from pulmonary disorders, the *basal respiratory efficiency is usually normal or subnormal*.

REFERENCES

- 1 Ziegler, E. E. New Measurement of Oxygen Absorbing Power, *M. Ann. Dist. Columbia* 2: 225, 1933.
- 2 Dautrebande, L. Le déficit cardiaque dans l'anémie, *Compt. rend. Soc. de biol.* 93: 1029, 1925.
- 3 Hurtado, A., Kaltrieder, N., and McCann, W. S. Respiratory Adaptation to Anoxemia, *Am. J. Physiol.* 109: 626, 1934.
- 4 Lijestrand, G., and Stenstrom, N. Work of Heart During Rest, Influence of Variations in Hemoglobin Content of Blood Flow, *Acta med. Scandinav.* 63: 130, 1925.

RECENT ADVANCES IN ENDOCRINE DIAGNOSIS AND TREATMENT*

JAMES H. HUTTON, M.D., CHICAGO, ILL.

WHILE endocrine disturbances frequently confront every doctor, not every patient should be subjected to an endocrine survey. The following conditions should lead to an endocrine examination:

1. Cases where the diagnosis is uncertain and not well supported by clinical and laboratory data.
2. Cases where the diagnosis seems correct but the indicated treatment fails to produce the results the doctor has reason to expect.
3. Every case of neurasthenia and nervousness and cases where there is uncertainty as to whether the patient has Graves' disease. While this syndrome is undoubtedly due to some endocrinopathy beyond the thyroid and probably will shortly be removed from the list of surgical diseases, at this time most men should merely make certain that there is not some other endocrinopathy responsible for the patient's complaints.
4. Growth deficiencies.
5. Genital hypoplasia.
6. Menstrual disorders not clearly due to local pathology in the pelvis.
7. Headaches.
8. Obesity.
9. Certain bone conditions, such as cysts, decalcification, slipping of the epiphyses, and fractures that fail to unite.
10. Diabetes mellitus.
11. Essential hypertension.
12. Patients who complain of mental or physical retardation, loss of memory and lack of concentration.

The diagnosis of endocrinopathies should be based on:

1. The exclusion of nonendocrine conditions.
2. A detailed history of the patient's complaints and of their relation to heredity, infection, surgical operation, or other trauma; also their relation to menstruation and pregnancy. The patient's developmental history should show, if possible, the weight at birth, whether the patient was delivered at full term, whether there was a likelihood of birth injury, whether the patient was bottle fed, the presence or absence of gastrointestinal upsets, and the age at which the teeth appeared and when the patient began to walk and talk. It is helpful also to know the course of the pregnancy at the termination of which the patient was delivered.

The menstrual history should disclose the age at onset, the interval between periods, the duration and amount of the flow and whether it has changed materially as to amount or duration; the location of pain and the time of occurrence, before, during or after the period; whether headaches are present and their relation to the periods: pituitary headache frequently occurs a few days before

*Delivered before the Peoria City Medical Society, June 18, 1935.

Received for publication, July 2, 1935.

the period, and whether the patient is the victim of colds at each menstrual period. If the patient has been pregnant, it is helpful to know the course of the pregnancies and how they terminated, whether by miscarriage or at full term. Habitual abortion is due to some endocrinopathy quite as often as it is to syphilis. One should also inquire whether the mother was able to nurse her children. Lactation is now known to be influenced by the anterior pituitary, and victims of anterior lobe deficiency frequently are unable to nurse their babies.

The history should also inquire into the height and weight of the relatives.

Physical Findings In addition to the routine careful physical examination, one should pay particular attention to the patient's stature, the proportion of the upper to the lower measurement and the relation of the span to the height, and to the condition of the skin and its appendages—the hair, nails and teeth. If obesity is present, its location should be noted, and the history should show whether it was acquired after an infection, such as measles or typhoid, after a surgical operation or after pregnancy. Pituitary obesity occurs under such circumstances.

Whether the anterior lobe makes as many hormones as it is sometimes credited with is doubtful, but it does elaborate a growth and a sex hormone between which there appears to be some antagonism, so that if the growth hormone is produced in superabundance, it may be at the expense of the sex hormone and vice versa. A youngster lacking a sufficient amount of the sex hormone may not only exhibit hypoplastic genitalia but also an increased growth of the long bones. This gives rise to the eunuchoid type of individual whose lower measurement (from symphysis to sole) may exceed by several inches the upper measurement (symphysis to vertex) and whose span may exceed the height. Other individuals lack the growth hormone but have a superabundance of the sex hormone. This results in well developed genitalia with a lack of growth of the long bones—an individual with short legs and a long body whose lower measurement may be much shorter than the upper.

Laboratory Data—In some instances this may need to be extensive. In others but little may be required. Most endocrinopathies exhibit lymphocytosis. This is most marked in thyroid deficiency. An increase in the eosinophiles is commonly present in pituitary deficiency and is less marked in adrenal and ovarian insufficiency. The blood sugar is usually low in pituitary deficiency, and the glucose tolerance curve is usually low, returning to the fasting level or slightly below at the end of four hours. The urea is said to be increased in adrenal insufficiency except late in Addison's disease. This is probably not often well enough marked to be very helpful. The uric acid is usually increased in pituitary deficiency. The calcium gives some help in studying the level of parathyroid function.

The x-ray is helpful in determining the bone age and the presence of bone cysts and of decalcification in hyperparathyroidism or pituitary basophilism. In studying bone age, various joints need to be x-rayed at different ages. The x-ray is one of our most useful implements in deciding whether a child under six is a victim of thyroid deficiency at ages when the basal metabolic rate gives unreliable information.

The visual fields are frequently contracted in the presence of pituitary deficiency.

Maliner's test consists of the injection of 1 c.c. of adrenalin. This is said to accentuate heart murmurs, to localize them more definitely to the areas over which they should be heard, and to develop murmurs not usually heard.

The insulin test was suggested by Harrop and others for a study of early or mild Addison's disease. The intravenous injection of very small doses of insulin is followed in such cases by prolonged and sometimes violent hypoglycemic attacks, which respond very indifferently to glucose either by mouth or by vein. The doctor who contemplates using this test should have his patient in the hospital.

Withholding table salt from the patient may bring about considerable aggravation of his symptoms if they are due to adrenal insufficiency.

The basal metabolic rate is always helpful. We have, unfortunately, believed that it reflected only thyroid function; that a low basal metabolic rate was caused or accompanied by a low thyroid function; but both pituitary and adrenal insufficiency may be responsible for very low basal readings. If a patient is found to have a low basal metabolic rate and few signs of thyroid deficiency, a search should be made for signs of pituitary or adrenal insufficiency. This is particularly true when the patient cannot tolerate even moderate doses of thyroid.

The signs and symptoms of hypothyroidism are too well known to need repetition here. It is an extremely common condition. It is best treated by the use of desiccated thyroid rather than by thyroxin for the reason that the body's response to thyroid can be more nearly controlled. It should be remembered that thyroid increases the heart's work before it increases its strength so that patients with severe degrees of thyroid deficiency are apt to feel worse after treatment is instituted. They may experience a good deal of palpitation, precordial distress, nervousness, and a trembling feeling. In such instances the thyroid should be stopped for a few days and then resumed in smaller doses or the patient should be put to bed for a few days.

Some relatively new endocrine syndromes have been described. In 1932 Cushing¹ described pituitary basophilism. This is characterized by vascular hypertension, hyperglycemia, frequently glycosuria and many times hypercalcemia. There is a rapid gain of painful obesity which is limited largely to face and trunk. There is an increase in hair growth. The menstrual periods usually cease. Flame-shaped purplish lineae atrophicae are noted in the groin; ecchymotic spots appear on the legs and the skin becomes peculiarly mottled. Frequently the patient complains of backache and pains in the joints, precordial distress, and difficulty in breathing. Wilder and others² have reported similar syndromes associated with adenoma of the adrenal cortex and so the picture is not so distinct as was thought at first.

Hyperparathyroidism is usually due to tumor or hyperplasia of one or more of the parathyroids. It is characterized by a disturbance of calcium metabolism. Calcium is usually present in large amounts in the blood stream and is excreted in large amounts in the urine. It is abstracted from the bones with their resultant rarefaction and softening. Cysts and tumors frequently occur. The

victim decreases in height and sometimes becomes a twisted gargoyle like caricature of his former self. Calcium deposits in the kidneys are common, polyuria and polydipsia occur, and much pain is complained of in the bones and joints. Treatment consists in removal of the tumor or one or more hyperplastic parathyroids.

Hyperinsulinism was first described by Seale Harris.³ Its symptoms are those of insulin shock. Epileptoid seizures frequently occur, and many patients have been diagnosed as epileptics. A low blood sugar is found during these attacks. There is doubt in my mind that hyperinsulinism is always at fault in these syndromes, although a few well authenticated cases are on record where a pancreatic tumor was found and removed with cure of the syndrome. In other cases, however, the patient was best relieved by doses of pituitary or suprarenal by mouth or hypodermically. At autopsy the pancreas was found to be a third the normal size and architecturally appeared to be normal. Even if the doctor prefers to call these cases hyperinsulinism, he should at least look for pituitary or adrenal deficiency.

Adrenal hemorrhage has been thought of as something recognized either at the autopsy table or shortly before the patient arrived at that point. However, Goldzieher and Gordon⁴ feel that adrenal hemorrhage in infancy should be recognized during life and that many patients can be successfully treated. The picture they draw of this condition is that of pneumonia plus a petechial rash on some part of the body, usually the extremities, and without the lung findings characteristic of pneumonia.

Adrenal Hypertension—It seems now fairly definitely established that some cases of paroxysmal hypertension are associated with tumors of the adrenal medulla or of similar tissue situated elsewhere in the body. Constant hypertension is said to be more often associated with hyperplasia or tumor of the adrenal cortex.

New Hormones—Preparations of the sex and growth hormone of the pituitary are now available. The experimentalists also use the thyrotropic, the adrenotropic, the diabetogenic and the fat metabolism hormone from the anterior lobe. Riddle and his workers have isolated a substance from the anterior lobe which is influential in lactation. They call it prolactin. Injected into laboratory animals it not only brings about lactation but also changes the emotional reaction so that virgin rats manifest maternal instincts. A pigmentation hormone is claimed for the pars intermedia. The testicular hormone has been isolated and identified.

New Therapeutic Agents—The parathyroid hormone is now available commercially. It is well standardized. A preparation of the hormone of the adrenal cortex essential to life is marketed under the name of eschatin. It should not be forgotten however that the adrenal cortex probably elaborates other hormones. At any rate the adrenals are concerned in the metabolism of sulphur, calcium, pigment, and sodium, tissue respiration and carbohydrate metabolism.

An insulin free extract of the pancreas is marketed under a number of names: padutin, angioxal, and kallikrein. It is useful in the treatment of angina pectoris. It has some value in the relief of pain and in increasing the blood flow through the extremities in various circulatory disorders. It is said to be useful in essential hypertension.

The sex hormone of the anterior lobe, or something that closely resembles it, can be separated from the urine of pregnant women and also from the placenta. Preparations of it are marketed under the trade names antuitrin-S, follutein, A.P.L. and progynon. These are useful in the treatment of amenorrhea, oligomenorrhea, and menorrhagia. For scanty or absent periods small doses are used; for the control of functional uterine bleeding larger doses are necessary, 2 or 3 c.c. daily. These preparations are of no value in the treatment of menopausal disorders. They are extremely valuable in the treatment of frigidity and sexual infantilism. They are also effective in the treatment of cryptorchidism.

Diathermy is recommended in the treatment of menstrual disorders associated with thickening of the ovarian tunic. The functional level of the adrenals and the pancreas is said to be raised by diathermy applied to those regions.

The parathyroid hormone is specific in the treatment of parathyroid tetany. It is also useful in the treatment of some forms of colitis, particularly the mucous variety, and in a syndrome which closely resembles, if it is not identical with, sprue. The French claim to have noted improvement in peptic ulcer following its use, and some cases of arthritis have been freed from pain by it. Some gynecologists report the control of menorrhagia by its use, and some cases of urticaria are relieved by it.

Various preparations of the ovarian hormone are available, such as theelin, amniotin, theelol, etc. These are particularly useful in disturbances of the menopause, not only the vasomotor phenomena but also the gastrointestinal complaints and the mental and emotional upsets occurring in this connection. At the Elgin State Hospital we treated seventeen women suffering from ovarian insufficiency. Ten of these improved to the point where they were able to leave the institution. Larger series with as good or better results have been reported by other men.

While the testicular hormone has been isolated, preparations of it are not available for the clinician. Probably the nearest approach to it is the testicular emulsion made by Stanley. It is not marketed but through the kindness of Dr. Stanley we were supplied with enough to treat three dementia precox cases in the Elgin State Hospital. Two of these very promptly improved to the extent that they were able to leave the hospital. Undoubtedly there is a close relationship between emotional and mental stability and the supply of the gonadal hormones.

For some years so-called light or stimulating doses of the x-ray have been applied to the pituitary and ovaries for the treatment of amenorrhea, dysmenorrhea, and menorrhagia. Many of these conditions were relieved and no bad effects were reported. Later Cushing used it in a few cases of pituitary basophilism. More recently we have used it in the treatment of diabetes mellitus and essential hypertension. Of hypertension alone we have treated 110 men and 54 women. Of these, 62 men and 35 women experienced some improvement. Nineteen men and 6 women were not improved. Twenty-five men and 8 women had an insufficient amount of treatment and 4 men and 5 women could not

be followed. We have had 18 patients who had both hypertension and diabetes, 13 men and 5 women. Of these 7 experienced improvement in both conditions, in 4 the hypertension alone was improved without influencing the diabetic condition, in 3 the hypertension was not affected but the diabetes was improved, the others either had an insufficient amount of treatment or could not be followed.

Barnes,⁵ working in Carlson's laboratory, removed the pancreas from ten dogs. These totally diabetic dogs, after being adjusted as to their diet and insulin requirement, were subjected to x-ray treatment applied to the pituitary and adrenals, following which their insulin requirement declined 50 per cent. These dogs were treated in pairs and the results in each pair were practically identical with those in every other pair.

Believing that O'Hare⁶ was right in suggesting that patients exhibiting a diabetic type of sugar curve during a glucose tolerance test are victims of essential hypertension, we have performed 63 tests on 61 hypertensive patients. Forty one of these patients exhibited a rise in blood sugar to above 170. Glycosuria was rarely observed. Twenty nine of these were improved by treatment. However, of the 20 patients exhibiting a nondiabetic type of curve, 16 were improved. Therefore we have given up the idea that the glucose tolerance curve is of any prognostic value in this work.

In the beginning we used very small doses of the x-ray consisting of the following factors: 110 KVM, 10×10 cm portal, 1 mm aluminum and 0.25 mm copper filter, 50 cm skin target distance, 4 milliamperes and twenty minutes to each side of the pituitary and the adrenals. We were unable to escape the idea that if little is good more is better and so the dosage was gradually increased until in a few instances we used the following factors: 180 KVM, 10×10 cm portal for each side of the pituitary, 15×15 cm common portal for the adrenals, 1 mm aluminum and 0.25 mm copper filter, 50 cm skin target distance, 5 milliamperes, fifteen minutes to the pituitary and twenty minutes to the adrenals, giving 142.5 r units to the pituitary and 190 to the adrenals. Almost invariably patients experienced an unpleasant reaction to this dose consisting of headache, tinnitus, weakness, and sometimes nausea. In only one case was there any notable reduction in blood pressure. We are convinced that whatever good can be accomplished can be brought about by very small doses of the ray. Consequently we returned to the small dose and now use the following factors: 120 KVM, 10×10 cm portal for each side of the pituitary, 15×15 cm common portal for the adrenals, 2 mm aluminum filter, 50 cm skin target distance, 4 milliamperes, seven minutes and about 70 r units.

In the beginning treatment was given at intervals of once a week until six treatments were given unless the blood pressure returned to normal levels before this series was completed. Now if there is a substantial reduction in blood pressure after the first treatment, no further exposure is ordered until it is evident that the blood pressure will not decline further or until it begins to rise. Instead of an immediate fall in blood pressure after treatment, there is frequently a rise lasting seventy-two hours and then a considerable drop in pressure.

Patients should be seen frequently while they are under treatment. The roentgenologist should not be saddled with the entire responsibility for the treatment of these patients, but he should be certain that his machine is accurately calibrated so that he knows how many R units per minute are delivered by it. The doctor in charge should decide when treatment should be repeated and whether the dose should be increased or decreased. How permanent the results effected by this method of treatment are is a question yet to be answered.

REFERENCES

1. Cushing, Harvey: The Basophil Adenomas of the Pituitary Body and Their Clinical Manifestations (Pituitary Basophilism), *Bull. Johns Hopkins Hosp.* 50: 137, 1932.
2. Wilder, R. N., Kepler, E. J., Kennedy, R. L. J., Walters, Waltman, and Davis, A. C.: Suprarenocortical Syndrome and Pituitary Basophilism: Presentation of Three New Cases, *Proc. Staff Meet. Mayo Clin.* 9: 169, 1934.
3. Harris, Seale: Hyperinsulinism and Dysinsulinism, *J. A. M. A.* 83: 729, 1924.
4. Goldzieher, M. A., and Gordon, Murray B.: The Syndrome of Adrenal Hemorrhage in the Newborn, *Endocrinology* 16: 165, 1932.
5. Barnes, B. O.: Personal Communication.
6. O'Hare (mentioned by Herrick, W. W.): Hypertension and Hyperglycemia, *J. A. M. A.* 81: 1942, 1923.

30 NORTH MICHIGAN AVENUE

LABORATORY METHODS

A MODIFICATION OF THE GREENBERG TECHNIC FOR THE COLORIMETRIC DETERMINATION OF SERUM PROTEIN*

A S MINOT PH D, AND MARGARET KELLER, A B, NASHVILLE, TENN

VARIOUS colorimetric procedures have been described for the determination of serum proteins¹⁻³. These methods are all based on the assumption that under the experimental conditions described there is a constant relation ship between the amount of protein present and the chromogenic value of a given serum or solution of protein as measured by the molybdate tungstate reagent (phenol reagent). Although other chromogenic substances undoubtedly enter into the reaction the principal phenolic groups measured are presumably those of the tyrosine present in the protein molecule. The color obtained from a known amount of pure tyrosine is used for comparison and the chromogenic value is usually expressed as tyrosine †. Provided that the assumed constant relationship holds, the amount of any protein for which the tyrosine:protein ratio has been established can be calculated from the results obtained from the colorimetric determination of the tyrosine present. The wide discrepancies in the protein equivalents of 1 mg of tyrosine established by the various investigators quoted above indicate that the experimental conditions of the color development greatly influence the color obtained. This fact, however, would not necessarily preclude a constant relationship provided that under the prescribed conditions the liberation of chromogenic groups proceeded always to a definite and permanent end point.

The chief advantage offered by the modification of Greenberg³ over the older colorimetric procedures was the introduction of the use of the improved phenol reagent of Folm and Ciocalteu⁴. This reagent avoided the difficulties of turbidity which had been previously encountered and made it possible to adopt a procedure which led to a rapid development of a more intense color from a given amount of protein. The technic described by the earlier investigators was further simplified by the adoption of Howe's⁵ method for the fractionation of serum proteins. The procedure for production of the color as described by Greenberg required only the addition of 2 cc of 5 N sodium hydroxide to about 25 cc of a definite dilution of serum or of the albumin fraction at room temperature. Chromogenic groups were immediately made

*From the Department of Pediatrics Vanderbilt University Hospital

Received for publication July 10 1935

This work was aided by a grant from the Division of Medical Sciences of the Rockefeller Foundation

†For convenience throughout the rest of this paper we shall use without further qualification the term tyrosine to indicate the chromogenic value of a serum or protein fraction as measured colorimetrically by the phenol reagent.

available to produce a deep blue color with the phenol reagent. By making the resulting colored solution to a definite volume and comparing with the color similarly derived from a known amount of tyrosine, the tyrosine value of the unknown was determined. From a comparison of his colorimetric results with the protein content of the same serums as determined by the Kjeldahl method, Greenberg derived factors which, under the described conditions, were assumed to hold generally for the calculation of the various protein fractions from their tyrosine content.

An attempt by us to apply this procedure and these tyrosine: protein factors to the routine determination of serum proteins gave results which at times varied widely from the figures obtained on the same serum by the Kjeldahl method. Our colorimetric results were at times too high and at other times too low. An investigation of the cause of these discrepancies convinced us that we were dealing with a rapidly progressing and, under the prescribed conditions, always incomplete reaction between the protein solution and the sodium hydroxide. Both the temperature of the solution at the time the sodium hydroxide was added and the time allowed to elapse between the addition of the sodium hydroxide and the phenol reagent greatly influenced the amount of color developed from the same amount of a given serum. The longer the time allowed for the reaction with sodium hydroxide and the higher the temperature at which it was carried out the greater the amount of color developed. These factors of time and temperature had so great an influence that even the range in room temperature encountered in this climate during a year made a great difference in the results obtained. Similarly at a given temperature the time allowed made so much difference that even a slight delay in adding the phenol reagent significantly increased the amount of color developed. In Table I are presented data which show the gradual increase in tyrosine values obtained on the same serum as the time allowed for the hydrolysis with sodium

TABLE I

EFFECT ON YIELD OF TYROSINE OF VARYING DURATION AND TEMPERATURE OF HYDROLYSIS WITH SODIUM HYDROXIDE*

TEMPERATURE ° C.	MG. TYROSINE PER 100 C.C. SERUM				
	2 MIN.	5 MIN.	10 MIN.	20 MIN.	30 MIN.
20°	426	441	468	482	503
25°	450	470	494	510	513
30°	460	482	510	519	530
35°	465	488	513	533	533
40°	491	516	530	540	548
45°	503	537	556	563	563
50°	522	556	560	580	584

*0.2 c.c. of serum + 20 c.c. H₂O + 4 c.c. 10 per cent NaOH used in every case. Same serum throughout.

hydroxide was lengthened at increasing levels of temperature. The results show that even after thirty minutes at 50° C. the chromogenic value is still increasing quite rapidly. Obviously no constant results could be obtained until conditions were worked out which would bring the reaction to a definite and constant end-point. To accomplish this the treatment with sodium hy-

dioxide was carried out in flasks immersed in a briskly boiling water bath. Although a strongly positive biuret reaction shows that the protein has not been completely hydrolyzed by this procedure, the figures presented in Table II indicate that a constant chromogenic value has been reached. On the five samples of serum studied the tyrosine values were constant after heating for periods from five minutes to one hour. Based on these observations the following procedures were adopted for the colorimetric determination of tyrosine in serum protein.

TABLE II

EFFECT ON THE YIELD OF TYROSINE OF CARBILING OUT HYDROLYSIS FOR VARYING LENGTHS OF TIME IN BOILING WATER BATH*

SERUM NUMBER	MG OF TYROSINE PER 100 CC SERUM					
	5 MIN	10 MIN	15 MIN	20 MIN	30 MIN	60 MIN
1	580	576	576	571	—	—
2	549	544	544	541	549	549
3	479	479	482	479	482	482
4	537	537	533	537	537	537
5	584	592	588	592	592	584

*0.2 cc serum + 20 cc H₂O + 4 cc 10 per cent NaOH used in every case

Total Protein—A one to ten dilution of the serum was made with physiologic saline. Two cubic centimeters of this dilution representing 0.2 cc of the original serum were placed in a 50 cc volumetric flask. Twenty cubic centimeters of water and 4 cc of a 10 per cent solution of sodium hydroxide were added. The flask was then placed in a boiling water bath for ten minutes. After cooling the contents of the flask thoroughly by standing it in ice water 3 cc of phenol reagent (Folin and Ciocalteu*) were added and the volume made to 50 cc with distilled water. A standard containing 0.8 mg of tyrosine in a volume of 50 cc was prepared in a similar manner. After allowing ten minutes for the color to develop comparison was made in a colorimeter. The standard tyrosine solution need not be heated with the alkali as no difference in the amount of color derived from pure tyrosine results from the procedure. For pathologic serums less concentrated standards containing 0.6 mg or even 0.4 mg of tyrosine may be necessary for satisfactory colorimetric comparison. When the usual 0.8 mg standard is used and the standard color is set at 20 mm in the colorimeter the calculation becomes

$$20/R \times 400 = \text{mg tyrosine per 100 cc} \quad R = \text{the reading of the unknown solution}$$

Even with the improved phenol reagent containing lithium sulphate we have noted a slight tendency for the solution to cloud if the laboratory temperature is very high unless the flasks are kept in ice water during the ten minute interval allowed for color development. Even a barely perceptible cloud influences the colorimetric readings in the direction of giving too high results.

Attention should be called at this point to the addition of water to the diluted serum before the sodium hydroxide is added. This was done in the determination of total protein and of albumin in order to make the dilution of alkali for the hydrolysis the same as was later found inevitable for globulin

It was found impractical to add larger amounts of sodium hydroxide to the protein dilutions because of the greater tendency for the colored solutions to cloud when the salt content was much increased. The 4 c.c. of 10 per cent NaOH which we have used is equivalent to the 2 c.c. of 5 N NaOH used by Greenberg.³

Serum Albumin.—The albumin and globulin fractions were separated by salting out the globulin with sodium sulphate according to the method described by Howe.⁵ The serum was diluted by adding twenty volumes of 22.5 per cent sodium sulphate. This results in a 1:21 dilution of the serum and a final concentration of 21.4 per cent sodium sulphate. After the addition of sodium sulphate the mixture was allowed to stand for several hours, usually overnight, in an incubator at 38° C. The globulin was then removed by filtration and the tyrosine content of the filtrate which contains the albumin was determined. Five cubic centimeters of the filtrate were placed in a 50 c.c. volumetric flask and 15 c.c. of water and 4 c.c. of 10 per cent NaOH added. The heating with the alkali and the color development with phenol reagent were then carried out exactly as has been described in the case of total protein. A standard containing 0.8 mg. of tyrosine in 50 c.c. gives a color suitable for comparison with the tyrosine obtained from the albumin of normal serums. More dilute standards may be necessary for pathologic specimens. The calculation using the 0.8 mg. standard set at 20 mm. is:

$$20/R \times 0.8 \times 2100/5 = \text{mg. tyrosine per 100 c.c.}$$

This may be simplified to

$$20/R \times 336 = \text{mg. tyrosine per 100 c.c.}$$

The amount of tyrosine obtained from the globulin in 0.5 c.c. of serum gives a color suitable for colorimetric reading. For the determination of globulin 0.5 c.c. portions of serum were diluted with 10 c.c. of a 22.5 per cent solution of sodium sulphate and allowed to stand as described above. The globulin was removed by filtration, washed with the Na_2SO_4 solution to remove all the albumin, and dissolved and washed into a 50 c.c. volumetric flask with approximately 0.01 N sodium hydroxide. The amount of solution necessary for washing was inevitably about 20 c.c. and this amount was always used. To this solution was added 4 c.c. of 10 per cent NaOH and the determination of tyrosine was carried out exactly as has been described. Again the 0.8 mg. tyrosine standard is usually suitable and when this is set at 20 mm. in the colorimeter the calculation is:

$$20/R \times 160 = \text{mg. tyrosine per 100 c.c.}$$

Before these values of tyrosine could be used for the determination of protein the tyrosine: protein relationship had to be established for these various types of protein under the newly adopted experimental conditions. To establish these ratios 25 samples of human serum have been studied. The tyrosine values of the various fractions were determined by the procedures

described above. For comparison with these results the proteins were determined on the same samples by the macro Kjeldahl method. The nonprotein nitrogen content of each serum was determined by the Folin and Wu method⁶ and this amount subtracted in the case of total protein and albumin from the total nitrogen determined by the Kjeldahl method. No nonprotein nitrogen is carried over into the globulin fraction so no correction is made in that determination. The protein nitrogen value was converted to protein by the generally accepted factor of 6.25. The tyrosine protein factor for the different types of protein was determined by dividing the milligrams of protein per 100 cc by the milligrams of tyrosine per 100 cc. Our results obtained on the 25 serums are presented in Table III.

It will be noted that in some instances the globulin factors were determined only by difference, while in the remainder of the samples they were determined

TABLE III
DETERMINATION OF TYROSINE PROTEIN FACTOR

SERUM	TOTAL			ALBUMIN			GLOBULIN		
	MG PER 100 CC		FACTOR	MG PER 100 CC		FACTOR	MG PER 100 CC		FACTOR
	PROTEIN	TYROSINE		PROTEIN	TYROSINE		PROTEIN	TYROSINE	
1	6300	497	12.67	3631	278	13.06	2669	219	12.19
2	7137	550	12.97	4481	356	12.59	2656	194	13.69
3	7025	543	12.94	4763	362	13.16	2262	181	12.50
4	7237	560	12.92	4750	366	12.98	2487	194	12.82
5	5476	440	12.45	3056	231	13.23	2420	209	11.60
6	7069	532	13.29	4720	358	13.18	2349	174	13.50
7	6833	510	13.39	4706	352	13.36	2127	158	13.47
8	7094	532	13.33	4700	352	13.35	2394	180	13.30
9	7257	537	13.51	4770	354	13.47	2487	183	13.58
10	6694	505	13.25	4806	342	14.05	1888	163	11.59
11	7175	518	13.85	4612	333	13.85	2563	185	13.85
12	7094	526	13.48	4861	350	13.88	{2756*	184*	14.98*
							{2233	176	12.66
13	7206	525	13.72	5156	380	13.57	{2225*	150*	14.83*
							{2050	145	14.13
14	7446	569	13.08	4993	367	13.60	2453	202	12.15
15	6169	476	12.96	3125	234	13.35	{3156*	248*	12.76*
							{3044	242	12.58
16	6955	540	12.88	4894	361	13.55	{2342*	190*	12.33*
							{2061	179	11.51
17	7200	558	12.91	4986	371	13.44	{2394*	188*	12.74*
							{2214	187	11.84
18	7343	567	12.95	5019	369	13.60	{2418*	190*	12.73*
							{2324	198	11.74
19	7737	586	13.20	5244	379	13.84	{2512*	190*	13.23*
							{2493	207	12.04
20	6737	503	13.39	4919	376	13.11	{2088*	159*	13.13*
							{1818	127	14.31
21	6912	508	13.60	4600	343	13.62	{2550*	197*	12.94*
							{2237	165	13.49
22	6887	522	13.19	4762	355	13.41	{2187*	155*	14.11*
							{2125	167	12.72
23	6600	506	13.04	4675	341	13.71	{2237*	-	-
							{1925	165	13.56
24	5450	417	13.07	4325	318	13.60	1125	99	11.36
							{2137*	166*	12.87*
25	6575	500	13.15	4362	327	13.34	{2213	173	12.80
	Average		13.25	Average		13.43	Average (11)		13.27*
							Average (25)		12.76
							Grand average		12.93

*By determination. All other globulins by difference.

both by difference and by direct determination. The factor by difference was derived from the ratio between the total protein minus the albumin protein and the total tyrosine minus the albumin tyrosine. There is greater individual variation in the factors obtained for globulin than for those determined for either total or albumin protein. This is true whether the figure was derived by difference or by direct determination. When determined directly there is

TABLE IV

COMPARISON OF RESULTS OBTAINED BY COLORIMETRIC METHOD X AVERAGE FACTOR AND BY MICRO-KJELDAHL METHOD. RESULTS GIVEN IN PERCENTAGE OF PROTEIN

NO.	MICRO-KJELDAHL METHOD			COLORIMETRIC METHOD		
	TOTAL	ALBUMIN	GLOBULIN	TOTAL	ALBUMIN	GLOBULIN
1	6.86	4.21	2.15	6.42	4.53	1.89
2	6.56	4.24	2.26	7.02	4.37	2.65
3	7.23	4.57	2.66	7.32	4.67	2.65
4	7.15	4.79	2.36	7.10	4.78	2.32
5	5.19	2.53	2.66	5.60	2.58	3.02
6	7.11	5.01	2.10	7.06	5.31	1.75
7	7.30	4.63	2.67	7.16	4.64	2.52
8	7.43	4.60	2.83	7.52	4.70	2.82
9	6.91	4.71	2.20	6.98	4.75	2.23
10	6.22	3.89	2.33	6.42	4.07	2.35
11	7.53	4.35	3.18	7.80	4.38	3.42
12	6.63	4.51	2.12	6.62	4.45	2.17
13	6.90	3.69	3.21	7.07	3.72	3.35
14	6.12	4.53	1.59	6.20	4.43	1.79
15	6.56	4.60	1.96	6.68	4.75	1.93
16	6.56	3.88	2.74	6.62	3.96	2.66
17	6.64	4.59	2.05	6.69	4.60	2.09
18	6.52	4.44	2.08	6.86	4.48	2.38
19	6.80	4.73	2.07	6.90	4.70	2.20
20	7.04	5.06	1.98	6.96	5.10	1.86
21	7.10	4.69	2.41	7.54	4.93	2.61
22	6.20	3.00	3.20	6.31	3.18	3.13
23	7.12	4.71	2.41	7.16	4.84	2.32
24	7.09	4.76	2.33	7.40	4.98	2.42
25	7.40	4.69	2.71	7.52	4.96	2.56
26	7.71	5.24	2.47	7.77	5.10	2.67
27	6.77	4.78	1.99	6.67	5.05	1.62
28	6.91	4.60	2.31	6.74	4.61	2.13
29	6.89	4.60	2.29	6.92	4.76	2.16
30	6.67	4.62	2.05	6.70	4.58	2.12
31	5.57	4.34	1.24	5.52	4.27	1.25
32	6.83	4.57	2.26	6.62	4.40	2.22
33	5.70	3.79	1.91	5.79	3.80	1.99
34	6.82	4.61	2.21	7.06	4.65	2.41
35	7.40	4.98	2.43	7.62	4.98	2.64
36	6.48	4.29	2.19	6.83	4.12	2.71
37	7.10	4.70	2.40	7.46	4.64	2.82
38	5.82	4.30	1.52	5.61	4.43	1.18
39	8.27	4.37	3.90	8.54	4.49	4.05
40	6.88	4.97	1.91	7.01	4.66	2.35

much greater chance for error because of the technical difficulty involved in the separation, filtration, washing, and solution of the globulin fraction than is met in either of the other two types of protein. When calculated by difference the variations in the total and albumin factors may be such as to combine to make a wider variation in the globulin factor. Furthermore, the so-called globulin fraction of serum protein is known to be made up of a varying mixture of proteins which may not all have the same chromogenic capacity. At

any rate, although it is of interest to know what the factor for globulin is, the figure is of little use since in practice the globulin in serum is almost universally calculated by subtracting the percentage of albumin from the percentage of total protein. As shown in Table III the average protein equivalents of 1 mg of tyrosine are as follows:

Total protein = 13.25 mg Albumin = 13.43 mg Globulin = 12.93 mg

Having derived these factors the next step in our investigations has been to test their reliability as compared with the Kjeldahl method for the determination of serum protein. Parallel determinations have been run on a series of 40 serums by our modified colorimetric technique and the micro Kjeldahl method using the sulphuric phosphoric persulphate digestion mixture described

TABLE V

COMPARISON OF COLORIMETRIC RESULTS & AVERAGE FACTOR WITH MICRO KJELDAHL'S DONE IN MEDICAL LABORATORY. RESULTS ARE GIVEN IN PERCENTAGE OF PROTEIN

SERUM	MACRO KJELDAHL			COLORIMETRIC X AVERAGE FACTOR		
	TOTAL	ALBUMIN	GLOBULIN	TOTAL	ALBUMIN	GLOBULIN
1	5.87	2.77	3.10	5.95	2.45	3.40
2	6.46	3.76	2.70	6.30	3.57	2.73
3*	1.50	-	-	1.49	-	-
4	6.35	4.54	1.81	-	4.39	-
5	5.79	2.76	3.03	5.58	2.46	3.12
6	6.98	4.14	2.84	6.84	3.97	2.87
7	5.61	3.76	1.85	5.41	3.52	1.89
8*	1.62	0.87	0.75	1.66	0.73	0.93
9	6.04	2.76	3.28	6.30	-	-
10	6.70	3.93	2.77	6.66	-	-
11	5.74	2.23	3.51	5.83	-	-
12	6.85	4.43	2.42	6.82	-	-
13*	3.07	2.07	1.00	3.24	-	-
14†	1.30	0.59	0.71	1.38	-	-
15*	1.34	0.71	0.63	1.34	-	-
16	5.72	2.17	3.55	6.14	2.03	4.12
17	6.83	4.37	2.46	6.35	4.30	2.05
18	0.71	4.66	2.05	6.59	4.30	2.29
19	7.97	4.13	3.84	8.09	3.82	4.17
20	4.07	2.44	1.63	4.09	2.32	1.77

*Ascitic fluid

†Pleural fluid

by Van Slyke.⁷ These serums were samples sent routinely to this laboratory for serum protein determinations. Some contain normal amounts of protein and others are quite pathologic specimens. The small volume of the samples available from infants and children made it necessary to substitute the micro for the macro Kjeldahl method in this series. A large number of parallel determinations has, however, shown good agreement between these two variations of the Kjeldahl method. The data in Table IV show the comparative results obtained by the colorimetric and micro Kjeldahl methods. The percentages of globulin were determined by difference in both methods. The data presented are entirely unselected and represent all the parallel determinations we have made. In analyzing the agreement between the results obtained by these two methods, it must be borne in mind that both are micro methods and that the experimental error in each approaches 0.20 per cent protein, hence occa-

sional discrepancies as great as 0.40 per cent of protein are inevitable regardless of the accuracy of the factor used.

As a further test a similar series of comparisons have been made between the results of our colorimetric determination of serum protein and those obtained on the same serums with the macro-Kjeldahl method by workers* in an independent laboratory. These results are presented in Table V and show the same agreement as when both procedures were carried out in this laboratory. Included in this series are a few determinations on ascitic fluid and other transudates. It is interesting to note that the same factors are applicable to the determination of protein in material of this type as in blood serum.

From these data it appears that when tyrosine is determined in various types of serum protein by the procedure which we have described, values are obtained which bear a constant relationship to the amount of protein present. Under these conditions the protein equivalents for 1 mg. of tyrosine are 13.25 mg. for total serum protein and 13.45 mg. for serum albumin. A considerable series of parallel determinations show that the procedure and these factors give values for serum proteins which agree closely with the results obtained by the more laborious Kjeldahl method. Serum globulin is probably best determined by difference.

* * * * *

In a conversation with Dr. David Greenberg at the time this work was nearing completion we learned that he had encountered the same difficulties with his original technic and was working out a modification similar to the one we have proposed. Later Dr. Greenberg kindly sent us his manuscript (this JOURNAL 21: 431, 1936) and also read a preliminary report of our work.

It will be noted that the protein equivalents of 1 mg. of tyrosine determined by Dr. Greenberg are lower than those which we have found. A careful comparison of the procedures described in the two papers shows that Dr. Greenberg heats his total protein and albumin fraction with a greater *concentration* of sodium hydroxide than we have used. The procedure for globulin appears to be the same as ours. A few experiments which we have carried out since the receipt of Dr. Greenberg's manuscript indicate that a somewhat greater chromogenic value is obtained by increasing the concentration of alkali. This would probably account for the lower factors at least in the case of total protein and albumin. If practical experience with the new procedure and factors presented by Dr. Greenberg show equally satisfactory agreement with results by the Kjeldahl method as we have obtained with the method which we describe there seems to be no particular advantage in one procedure over the other. The point must be stressed, however, that whatever method and factors are adopted for the colorimetric determination of serum proteins the prescribed experimental conditions must be closely followed.

*We wish to express our thanks to Mr. J. H. Akeroyd, Jr., of the Department of Medicine for allowing us to present the results of his Kjeldahl determinations and for furnishing the samples of serum for our parallel colorimetric determinations.

REFERENCES

- 1 Wu, H. A New Colorimetric Method for the Determinations of Plasma Proteins, *J Biol Chem* 51 2, 1922
- 2 Wu, H., and Ling, S. M. Colorimetric Determination of Proteins in Plasma Cerebrospinal Fluid and Urine, *Chinese J Physiol* 1 161, 1927
- 3 Greenberg, D. M. The Colorimetric Determination of the Serum Proteins, *J Biol Chem* 82 545, 1929
- 4 Folin, O., and Ciocatta, V. On Tyrosine and Tryptophane Determinations in Proteins, *J Biol Chem* 73 627, 1927
- 5 Howe, P. E. The Use of Sodium Sulphate as the Globulin Precipitant in the Determination of Proteins in Blood *J Biol Chem* 49 93, 1921
- 6 Folin, O., and Wu, H. A System of Blood Analysis *J Biol Chem* 38 81, 1919
- 7 Van Slyke, D. D. Gasometric Micro Kjeldahl Determination of Nitrogen, *J Biol Chem* 71 235, 1927

THE INFLUENCE OF THE COMPOSITION OF THE ANTIGEN
EXTRACTS FOR SOME SERODIAGNOSTIC TESTS ON
SYPHILIS (M B R II and M K R II)*

BERTH JOSEPHSON, M D DOCTOR STOCKHOLM, SWEDEN

SEVERAL investigators (Georgi, Vermast, Kahn, Meinicke, Laubenheimer, Eagle, Norel, Poeplau, and others) have pointed out that for the flocculation reactions on syphilitic blood there often is a fixed proportion between the concentrations of the suspended substance and the lipoids from the heart extract, at which the reaction turns out most advantageously.

Of the great number of flocculation tests used in the serum diagnosis of syphilis those of Kahn, Muller (M B R II) (see the *comparison of Moersch*) and Meinicke (M K R II) are commonly used in Sweden. The reagents for the tests of Kahn are well described in several publications. Not very exact accounts however, are given of the composition of the extracts for the tests of Muller and Meinicke. For this reason I have found it of interest to examine the influence of differences in the composition of the combined lipid solutions for these reactions.

In both the tests in question the antigenic lipoids are from alcoholic ox heart extracts. The suspended substance is in the M B R II cholesterol and in the M K R II tolu balsam. Eagle has tried to give an explanation of the fact, that the Wassermann and other similar tests become more sensitive with increasing cholesterol content in the antigen solution. Which was now the influence of alterations in the proportion between the concentration of the heart lipoids and the matters to be suspended in the two reactions mentioned above?

THE COGLOBATION REACTION OF MULLER (M B R II)

According to the information given by Muller the reagent used in this test is an alcoholic extract of ox heart (1:30). The extract would have to be concentrated on water bath and tempered with much cholesterol.

*From the Chemical Department of the Caroline Institute Stockholm

Oxhearts were discharged from fat and thoroughly ground. Eighteen hundred grams of the ground hearts were extracted with 15 liters absolute alcohol (Extr. 1), 800 gr. were extracted with 6 liters absolute alcohol (Extr. 2) and 200 gr. also with 6 liters (Extr. 3). Finally 180 gr. were treated with 1.5 liters alcohol (Extr. 4). For all the extractions the shaking machine was used three hours a day during three days. Extr. 1 was then stored for three months at about 22°-27° C. before filtering, Extrs. 2 and 3 for three weeks at room temperature, Extr. 4 for three weeks at 38° C. They then were filtered. Extr. 3 according to Müller's suggestion was concentrated on a water-bath to a third of the original volume and filtered again. The analyses of the filtered extracts gave the following values (Table I). N-analyses Micro-Kjeldahl, P-analyses Pregl.

TABLE I

EXTR. NO.	SPEC. GRAVITY 20° C.	CHOLESTEROL MG./ML.	DRY RESIDUE MG./ML.	ASH MG./ML.	N MG./ML.	P MG./ML.
1	8.33	0.15	6.75	0.67	0.88	0.155
2	8.30	0.13	4.94	0.66	0.25	0.147
3	8.42	0.19	6.43	0.77	0.27	0.169
4	8.36	0.19	5.54	0.54	0.25	0.111

By warming in 38° C. thermostat 0.55 per cent cholesterol was solved in parts of all the extracts. Each extract was then divided in parts and tempered with 3, 3.5, 4, etc. until 7 per cent ether per volume, respectively. After inoculation of some cholesterol crystals the solutions were left standing at 20° C. for two days. As shown by Vermast, the solubility of cholesterol in alcohol is very much dependent not only on the eventual presence of small amounts of water but also on the temperature. The solutions were filtered from crystallized cholesterol and the remaining cholesterol content again determined.

Each filtrate was then tried in the conglobation reaction (the modification with 0.10 and 0.15 ml., respectively) after the instructions given by Müller. A considerable number of Wassermann reaction positive and negative serums were used.

All the experiments with Extr. 3 (prepared after Müller) gave bad results. The precipitates with the positive serums were not clot-shaped and sunk to the bottom of the tubes. Therefore this extract was excluded. Furthermore, all the other extracts with more than 5 per cent ether tended to separate at the dilution with the salt solutions; and in the tubes with negative serums there appeared precipitates on the bottoms of the tubes or small clots ("snowing"). Generally the strongly positive serums with these extracts gave beautiful clots, which now and then had sunk to the bottom. The reaction was oversensitive and the cholesterol content too high.

The extracts with less than $\frac{1}{3}$ per cent ether, on the other hand, did not divide at the dilution. With all the negative serums they gave clot-free suspensions, which, however, often kept a little smeary precipitation. With some of the positive serums they showed no clotting, only a flocculation. The reaction was not very sensitive and the cholesterol content was too low.

Very good reactions with both the negative and the positive serums were obtained by using Extrs. 1 and 4 with 4 per cent ether, and Extr. 2 with 3 per cent ether. In these samples there was found for Extr. 1, 0.53 per cent cholesterol, in Extr. 2, 0.39 per cent, and in Extr. 4, 0.45 per cent. These fractions

gave very beautiful reactions, especially in the case of Extr 1, but also of Extr 4. The clots observed in syphilitic serums with Extr 2 were sometimes a little thin and ramified.

Now big quantities of the three solutions just mentioned were prepared and tried on usual serums and cerebrospinal fluids, sent in to the serologic laboratories for testing for syphilis.

Extracts, made as number 1, have been used in about 20,000 cases, of which about 1,000 were positive. In all cases the Wassermann reaction also was made in various forms, and in many of them Kahn's standard test and Meimcke's M K R II as well. On about 3,000 of these serums the clotting test of Muller also has been carried out at the same time with the original German "Ballungs reagens." In all cases the results with my extract have shown an extremely good correspondence with the other reactions and with the informations from the clinics. In the same way and with nearly similar results Extr 2 has been tested on about 500 samples and Extr 4 on about 1,200.

Thus the best way to prepare this reagent seemed to be the one used for Extr 1 or possibly Extr 4 with 3 or 4 per cent ether.*

The reactions seemed to be more clear and selective when the cholesterolized extracts had been left to ripen for three to five months.

It must be mentioned that Poeplau has made an investigation of the cholesterol content suitable for this reaction and found it very definite. He also preferred the nonconcentrated extracts. However, he seems to have tried only one single heart extract, and he does not mention any analyses. He concluded that the reagents were oversensitive when there was too little cholesterol.

THE CLARIFICATION TEST OF MEIMCKE (M K R II)

Meimcke states that the reagent for his M K R II consists of an alcoholic extract of ground oxheart, which has been dried at 56° C, extracted with ether and dried again. The reagent should also contain 14 per cent tolu balsam and 0.01 per cent victoria blue. The latter figures are also found in works by Boas and Izikowitz. I have not been able to find any exact description for the preparation of the heart extract for this, the latest reaction of Meimcke.

Oxhearts were well ground dried at 56° to 58° C on glass plates and ground again. Three hundred grams of the powder were extracted for five days with ether, the first day by aid of machine shaking. Ten milliliters ether were used for each gram of the powder. The ether was filtered off and the residue again extracted with the same amount of ether. After drying at 37° C one third of the powder was extracted with 0.9 liters of alcohol 95 per cent.

TABLE II

EXTR.	DRY RESIDUE MG / ML	ASH MG / ML	N MG / ML	P MG / ML
A	9.59	1.025	0.75	0.238
B	5.02	0.518	0.42	0.121
C	15.68	1.619	1.04	0.419

(Extr A), one third with 2 liters (Extr B) and one third with 0.5 liters (Extr C), respectively. Also this extraction continued for five days, the first one in the shaking machine. The analyses are shown in Table II.

*Extract of the Type 1 is now kept for sale by The Astra Ltd Södertelje Sweden.

Now dilutions with alcohol were made of these extracts containing 5 per cent, 10 per cent, 20 per cent and so on, of the extract. Each of these solutions, again, was divided in parts, in which was dissolved 1.0 per cent, 1.1 per cent, 1.2 per cent, etc., of tolu balsam. To all of them there finally was added 0.002 per cent victoria blue (Herzberg-Hollborn).

These solutions were tested in the "Klärungsreaktion" on positive and negative serums. The instructions given by Meinicke were scrupulously followed. Between those samples, which seemed to give the best results, closer dilutions were made. It turned out that as already pointed out by Meinicke the best reactions were obtained by using a definite proportion between the tolu balsam content and the content of solids from the original extract. With this proportion the different extracts showed similar activity. At low concentrations, 1.1 to 1.6 per cent balsam, this proportion was about 13, but at increasing concentration (2 to 3 per cent) it was somewhat lower, about 8 to 10. If there was too little balsam, the negative tests remained homogeneous, but the positives showed a turbidity, increasing with decreasing balsam concentration until the reaction seemed negative. The extract was not sensitive enough. A decrease of the heart extract concentration, again, caused an increasing clarification of the negative samples, the positive remaining manifest. It was too sensitive. The allowed limits for the proportion seemed to be much more narrow at low concentrations than at high.

Altogether the reaction was more distinct at rather low concentrations, the best reagents containing 1.2 to 1.3 per cent balsam and 0.09 to 0.10 per cent heart solids. The test then turned out very clear. The results with the different extracts were closely corresponding.

The concentration of victoria blue may be varied within wide limits. Using the Herzberg preparation from Hollborn, which proved to be the best of several tested, the ideal concentration was 0.0015 to 0.0020 per cent. (Meinicke directs 0.01 per cent.)

Five such extracts of Type A* were prepared from different hearts and their reacting compared with the other syphilis tests as for the conglobation reagent. These extracts all reacted similarly. Comparison was also made with the original German M.K.R. II extract. Until now about 10,000 cases (about 200 cerebrospinal fluids) of which about 900 positives have been examined with the new extracts, have all given results corresponding well with those found with the other reactions, and also with the reaction carried out with the original M.K.R. II reagent from the "Adler-Apothek" in Germany. In all cases the macromodification of Meinicke was used. In some cases also the micro-modification was tested.

SUMMARY

An investigation of some different reagents for the conglobation reaction of Müller (M.B.R. II) and the clarification reaction of Meinicke (M.K.R. II) is described. It is shown, that for these reactions the proportion between the oxheart lipoids and the substance, to form the suspension (cholesterol, tolu balsam) must be of a definite magnitude. Too much heart lipoids will make the reaction less sensitive and too little the reverse. Confirming the theory of

*This extract now is kept for sale by The Astra Ltd, Södertelje, Sweden.

Eagle, this may be due to a lability in the colloidal antigenic suspension. The less of the charging heart lipoids, the easier the suspension will be discharged and precipitated. The proportion ought to be tested out for each heart extract. The conglobation reagent for the Muller test ought to contain 3 or 4 per cent ether to keep sufficient cholesterol (0.50 to 0.55 per cent) in solution. For the M K R II the proportion between tolu balsam and heart lipoids should be about 13 and the total concentration 1.4 per cent. The convenient concentration of the suspension system must within certain limits be determined and fixed.

It is a pleasure to me to express my thanks to Docent H. Davide for his kindness in helping me to try my extracts in his serologic laboratory. I also wish to thank Miss Daldqvist who has assisted me in the testing of the extracts.

REFERENCES

- Boas, H. Finska Läkarsällskapets handlingar 75: 439, 1911.
 Eagle, H. J. Exper. Med. 52: 717 and 747, 1930. J. Immunol. 23: 153, 1922.
 Georgi, W. Ztschr. f. Immunitätsforsch. u. exper. Therap. 27: 315, 1915.
 Izikowitz, S. Svenska Läkartidningen 71: 652, 1934.
 Kahn, R. L. Serum Diagnosis of Syphilis. Baltimore, 1925, The Kahn Test, Baltimore, 1925.
 Lubbenheimer, K. Handb. d. path. Mikroorg. 7: 1, 216, 1930.
 Memcke, E. München. med. Wchnschr. 79: 375, 1932, Zentralbl. f. Bakt. 125: 356, 127, 187, 1932, Monography 1933, J. Lab. & Clin. Med. 19: 518, 1924. München. med. Wchnschr. 81: 100, 1934.
 Moersch, J. Quart. Bull. Health Org. League of Nations 1: December, 1932.
 Muller, R. Zentralbl. f. Haut u. Geschl. kr. 18: 492, 1926. League of Nations publ. III, Health 3: 133, 1929, Deutsche med. Wchnschr. 55: 1624, 1929.
 Norel, K. Comm. Inst. Sérother., Danvers 20: 31, 1930.
 Pocplau, P. Ztschr. f. Immunitätsforsch. u. exper. Therap. 66: 240, 1930.
 Vermyst, P. S. F. Ztschr. f. Immunitätsforsch. u. exper. Therap. 34: 95, 1922.

A NEW DILUTING PIPETTE FOR THE SAHLI HEMOGLOBINOMETER*

HELMUTH ULRICH, M.D. BOSTON, MASS.

THE pipette to be described is designed to be used with the Sahli hemoglobinometer. It has two distinct advantages: (1) It obviates the necessity of carrying the entire Sahli apparatus to the patient's bedside or home, (2) it permits making the estimation of hemoglobin at a convenient time subsequent to obtaining the blood.

In principle, it is similar to the diluting pipettes that are used for the estimation of the number of erythrocytes and leucocytes. It differs from the usual Sahli pipette only in having a bulbous dilatation above the 20 cmm mark. The capacity of the bulb is approximately equal to that of the Sahli tube to the mark 10. The 20 cmm mark is its only graduation. The initial dilution of the blood with hydrochloric acid is made in the pipette rather than in the Sahli tube.

Directions for Use—Draw the blood from the usual puncture wound of the ear or finger (or oxalated blood) exactly to the 20 cmm mark, then

*From the Evans Memorial of the Massachusetts Memorial Hospitals and Boston University School of Medicine.

Received for publication July 16, 1933.

aspirate sufficient tenth normal hydrochloric acid to fill the bulb. If the estimation is not to be made at once, close the openings of the pipette with a wide rubber band. When ready to proceed, eject the contents of the pipette into the Sahli tube and add water in the usual manner until the color of the diluted blood matches that of the standard.*

It is probably correct to state that many estimations of hemoglobin by the Sahli method are made without allowing sufficient time for the full development of the color of acid hematin. The usual directions that accompany Sahli outfits or are given in textbooks (if they are stated at all) specify that a few minutes should elapse before the comparisons are made. Nicholson¹ claimed that "95 per cent of the acid-hematin color" will develop in ten minutes, but according to Osgood² "the reaction . . . requires at least twenty-four hours at room temperature to become complete." They recommended

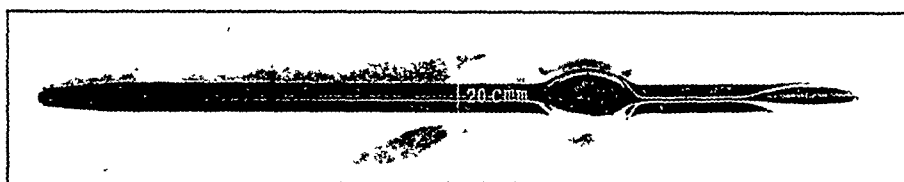


Fig. 1.

immersion of the tube containing the blood and acid mixture in a beaker of warm water (55°-60° C.), at which temperature "the reaction is completed in seven minutes." For practical purposes, one hour at room temperature appears to be sufficient, as is shown by the results obtained by the Sahli method in four cases (see Table I).

TABLE I
SHOWING THE EFFECT OF TIME ON THE COLOR OF ACID HEMATIN

CASE	READING					
	IMMEDIATE	1 HOUR	2 HOURS	6 HOURS	24 HOURS	48 HOURS
1	67%	72%	70%	71%	71%	70%
2	79%	83%	81%	84%	82%	
3	91%	100%	100%	101%	100%	100%
4	89%	93%	94%	92%	94%	

The minor variations recorded after the first hour cannot be ascribed to changes in the color, but must be accepted as errors inherent in colorimetric determinations. The essential but frequently neglected time interval is an additional point in favor of using the special diluting pipette.

REFERENCES

1. Nicholson, D.: *Laboratory Medicine*, Philadelphia, 1934, Lea & Febiger.
2. Osgood, E. E.: *Laboratory Diagnosis*, Philadelphia, 1935, P. Blakiston's Son & Co., p. 396.

99 BAY STATE ROAD

*The pipette and a hemacytometer case with an extra groove for it may be purchased on special order from Arthur H. Thomas Co., Philadelphia, Pa. Both will be established as catalogue and stock items, if a sufficient demand develops. The additional groove in the hemacytometer case may be used also for a spare red or white pipette and should prove convenient for those who use a white pipette for hemoglobin determinations by the Haden-Hausser method.

A SYNCHRONOUS MOTOR ELECTRIC TIME CLOCK*

A. L. BENNETT, PH.D., OMAHA, NEB.

THIS clock provides electrical contact at intervals of 1, 5, 15, 30, and 60 seconds as well as 5, 15, 30, and 60 minutes without adjustment of any clock contacts. Two switches provide simple and instantaneous setting for any of the intervals. Contact is short and uniform regardless of interval. The clock is accurate, dependable, and entirely adequate for timing research experiments or

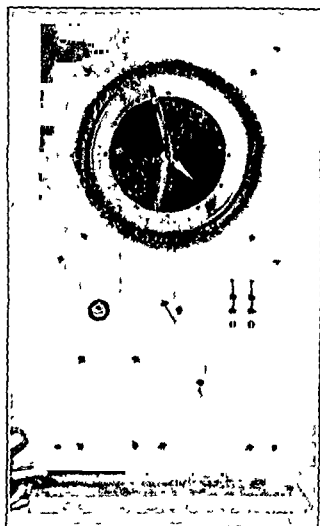


Fig 1

as a master clock for a general laboratory. Furthermore it may be easily made in a laboratory workshop at a very nominal cost.

A small synchronous electric clock motor is mounted with the central pinion extending through a panel of insulation material in such a way as to permit replacement of the hands and use of the panel as a dial for the clock. Contacts are inserted through the panel so that the "second" hand makes contact with the outer circle of sixty points while the "minute" hand reaches the inner circle of twelve points. All wiring is done on the back of the panel. The front

*From the Laboratory of Physiology and Pharmacology, University of Nebraska Medical School.

Received for publication, June 6, 1935

of the dial is polished so that the contacts are flush with the surface and the hands slide smoothly over metal and insulation material alike. Dimensions may be made to suit the particular clock motor which is used. Following are those used by the author:

Radius for circle of outer contacts	47.0 mm.
Radius for circle of inner contacts	42.0 mm.
Diameter of outer contacts	1.3 mm.
Diameter of inner contacts	2.9 mm.

The outer circle of dial contact points which furnish the "second" intervals from 1 to 60 are made of brass brads which are driven through the panel

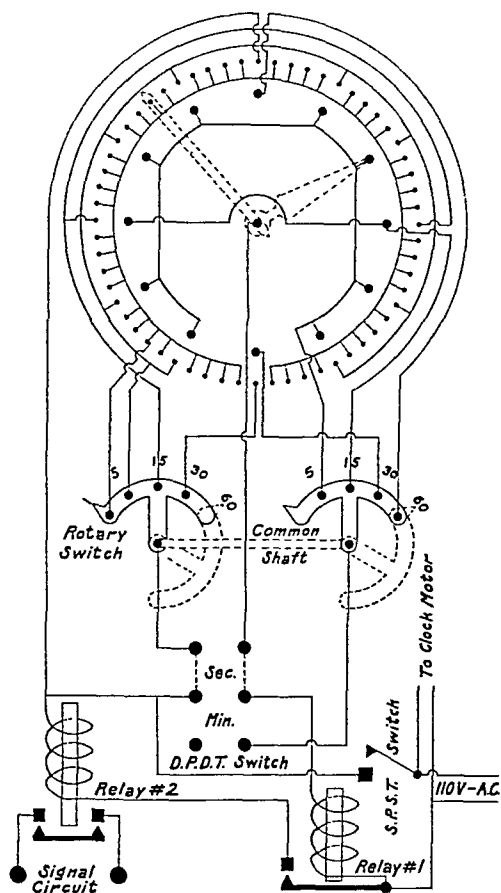


Fig. 2.—Diagram of circuit.

after it is drilled with holes that provide a very snug fit. The larger contacts of the inner circle, which provide the "minute" intervals from 5 to 60, are made of brass wood screws. These are turned into snugly fitting drill holes until the smooth section of the screw has entered the panel. The head is clipped off and then screws and brads alike are filed flush with the dial surface, leaving each projecting on the back of the panel for solder connections. The front of the dial must be polished carefully so that the hand contacts will pass over it

smoothly. This is best done after the soldering has been completed on the back of the dial, as otherwise the heat may roughen the surface already polished.

The hand contacts are made by cutting narrow strips from the two halves of a gold fountain pen point and soldering these to the "second" and "minute" hands of the clock. These points are adjusted so that the midium tips contact the brass points of the dial. The second hand moves more smoothly if the tip is made to follow the hand by about one fourth of an inch and is thus "dragged" over the dial surface. If the second hand is too flexible, it may be replaced with a strip of tin.

The main control switch is a double bank four contact, Varley radio switch. This is made accumulative rather than selective by replacing the single rotating contacts with strips of metal which will cover all four of the stationary contacts when the switch is turned to the stop toward the left. This provides in one control the selection of interval. The small double pole double throw radio switch determines whether the interval shall be in seconds or minutes. A standard radio toggle switch is used to close the relay circuit. Reference to the wiring diagram will make this clear.

In order to use the 110 V A C current to activate the relays as well as run the clock, a 10,000 ohm sensitive relay is placed in circuit with the clock contacts. This draws only 2.5 milliamperes and thus protects the clock contacts from burning and becoming rough. A second relay activated through the first has heavy enough contacts to carry the load of several signals if the clock is to be used as a master timer for a large laboratory.

Specifications for the materials used by the author:

Clock General Electric No AB762
 Rotary Switch Varley No 1226
 Relays Struthers Dunn Inc Philadelphia
 No 1 Type CXB51 10,000 ohm A C coil
 No 2 Midget Type ABTX A C coil
 Panel Insuline 7 x 12 x $\frac{3}{16}$ inches

If the above clock is used the sides and back of the case may be fastened to the back of the panel to provide protection for the motor and electric connections. The relays and switches may then be covered with a separate case. Since there is no adjustment necessary at the clock contacts a glass cover may be fastened tightly over the dial thus making it dust proof.

THE IMPORTANCE OF LEUCOCYTE COUNTS IN PHAGOCYTIC TESTS*

RUTH WESTLUND JUNG, B.S., M.S., PH.D., CHICAGO, ILL.

IN DETERMINING the opsonic index of an individual's serum by Wright's method the number of leucocytes is not important since the same leucocyte suspension is used for both the test and control serums. When, however, one compares the phagocytosis occurring in one sample of whole blood with that present in another the number of leucocytes should be taken into account. The importance of knowing the number of leucocytes used in phagocytic tests was suggested more than thirty years ago, yet some present-day investigators disregard this point in describing their "quantitative" tests for the phagocytic power of an individual's blood.

Dodd¹ in 1907 called attention to the discrepancy in Leishman's method of obtaining opsonic indices by comparing the amount of phagocytosis in the whole blood of an individual with that occurring in the blood of another person. He realized that the differing number of leucocytes in the blood was the cause and he attempted to remedy it by applying a simple formula as follows. Counts were made on the mixtures of bacteria and blood used in the test, employing a red blood cell counting pipette. To correct the apparent index he multiplied the control index, obtained by Leishman's method, by its corresponding number of leucocytes and divided the result obtained into the other index multiplied by its corresponding number of leucocytes. In this way he secured results approximating those obtained by Wright's method. Below is shown an example to illustrate the method.

	NO. BACTERIA PER 100 CELLS	LEISHMAN INDEX	WRIGHT INDEX	NUMBER OF LEUCOCYTES
Control A	190	1.0	1.0	11000
B	115	0.6	0.9	16000
	$\frac{0.6 \times 16000}{1.0 \times 11000} = 0.87 = \text{corrected index}$			

Fleming² in 1908 observed higher readings with fewer corpuscles. A five-fold dilution of the leucocytes caused a 50 per cent increase in the phagocytic index. Twofold dilution gave results 10 to 26 per cent higher than when undiluted blood was used. He considered the bacterial emulsion of the greatest consequence to accuracy and the leucocyte suspension next. Doubling the volume of leucocytes reduced the index to 0.8 while using one-half the volume increased it to 1.15. Doubling the volume of bacterial suspension increased the index to 1.33. Using one-half the volume decreased it to 0.68.

*From the Department of Bacteriology, Northwestern University Medical School.
Received for publication, June 28, 1935.

Hektoen³ in 1911 wrote that one can compare the phagocytic properties of leucocytes from a particular source with those of other leucocytes by finding the amount of phagocytosis by each only under strictly comparable conditions. For such purposes the phagocytic mixture which is the same in everything except the source of leucocytes self evidently must contain the same number of leucocytes. He suggested that a leucocyte count be made and that the suspensions be made comparable by dilution with NaCl. He adds that it is quite evident in mixtures containing an unequal number of leucocytes there will be less phagocytosis per phagocyte in a mixture containing larger numbers of leucocytes and vice versa. In other words an apparent difference in phagocytic power may result from an inequality in the number of leucocytes in the mixture studied. He cites the experiments of several investigators and notes their failure to take the number of leucocytes into consideration.

EXPERIMENTAL

The recognition of the importance of the leucocyte count, in the phagocytic test, by some of the earlier workers and yet completely ignored by some present day investigators led us to investigate this question further.

Since the present tendency seems to be to evaluate phagocytosis by the percentage of leucocytes containing bacteria or particles rather than the actual number of bacteria or particles contained in the cell, the findings reported in this article are on that basis. The present paper is an expansion and continuation of an earlier report.⁴

The question arose of how to obtain samples of blood alike in all respects except the number of leucocytes contained. Since no attempt was made to separate the leucocytes from the red blood cells, diluting with salt solution, which would reduce the number of leucocytes, would also change the number of red blood cells and the conditions in two samples of blood would not be comparable. A few experiments were conducted in this way, however, merely to give an indication of the relation of the number of phagocytes to the percentage phagocytosis. It can readily be seen from Table I that as the blood is diluted the percentage of phagocytosis increases.

TABLE I

DILUTION OF BLOOD	PERCENTAGE OF PHAGOCYTOSIS			
	EXPER 1	EXPER 2	EXPER 3	EXPER 4
Undiluted	32	43	40	36
1 2	43	48	47	41
1 3		58	71	53
1 4	53		72	74

To determine that the number of leucocytes was the responsible variable all other inconstants were removed by using deleucocyted blood as a diluting fluid, thus permitting the numbers of leucocytes to be varied without changing the red blood cell count. The blood was deleucocyted by a modification of the method of Fleming⁵. For counting the leucocytes it was necessary to find another counting solution since the usual 1 per cent acetic acid did not completely dissolve the heparinized or citrated washed erythrocytes. These appeared in large clumps and interfered seriously with the leucocyte count. Experiments

designed to obviate this difficulty indicated that the use of heparin or citrate was not responsible but that centrifuging and washing with salt or Tyrode's solution caused this phenomenon. The addition of 0.8 per cent CaCl_2 to the 1 per

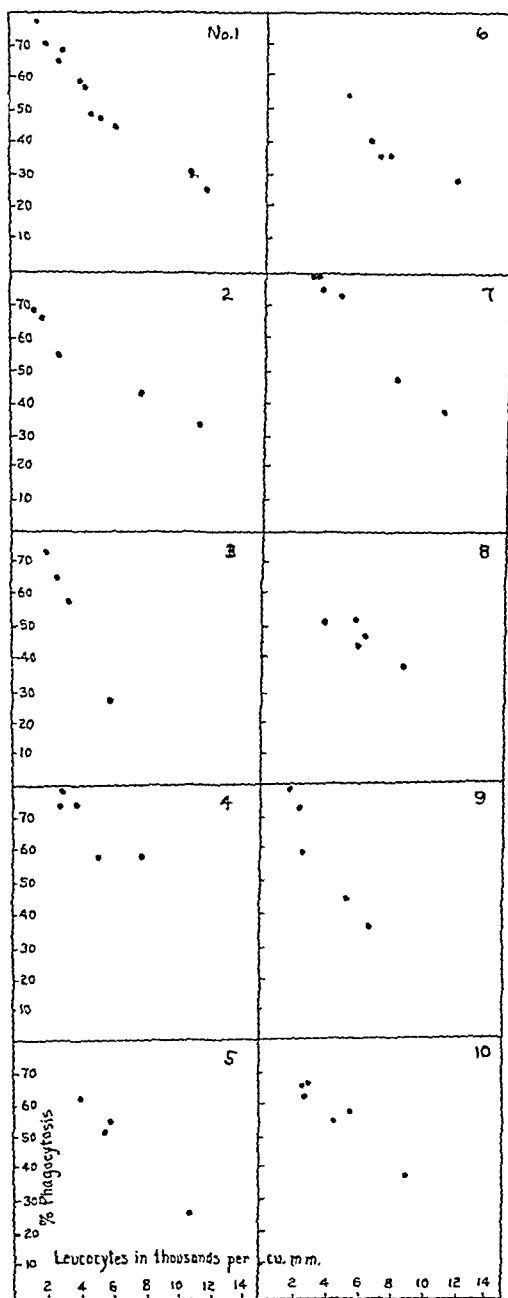


Chart 1.—Correlation between leucocyte count and percentage of phagocytosis in ten normal human male adults.

cent acetic acid solution lessened the difficulty to a great extent and was used in the earlier experiments. A 0.5 per cent HCl solution was subsequently found

more satisfactory and was employed in the later work. It is essential, however, that the leucocytes be counted as soon as mixed with the counting fluid since the HCl has a tendency to dissolve the leucocytes if left standing for any length of time. Counts of leucocytes on uncentrifuged blood with 1 per cent acetic acid and 0.5 per cent HCl were comparable.

After preparing samples of blood containing varying numbers of leucocytes phagocytic tests were run using a heat killed culture of streptococcus and serum from the blood of the individual tested. The usual capillary tube method was employed. The results showed that this method was as accurate for this purpose as the more recently adopted methods of keeping the materials in agitation during incubation of the test. Fifteen minutes at 37° C. was chosen as the incubation time. While the leucocyte count made on the heparinized blood is not an exact evaluation of the number used in the test it gives the relative numbers in each sample of blood, and this is the important point.

On plotting leucocyte concentrations as abscissae and percentage phagocytosis as ordinates it was clear in every instance that the two quantities were

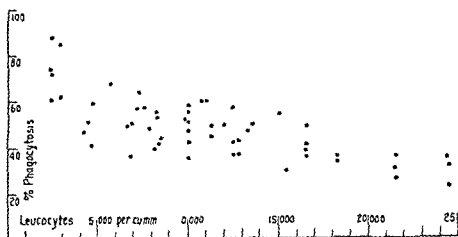


Chart 2—Correlation between leucocyte count and percentage of phagocytosis in ten normal dogs

related. Fifty-six determinations were made on 10 normal male adults and 56 on 10 normal dogs. The relation of percentage phagocytosis to the number of leucocytes appeared to be linear so that for a given subject the data could be represented by a line whose slope was equal to $\frac{y_2 - y_1}{x_2 - x_1}$ where x = the number of leucocytes and y the percentage of phagocytosis. The slope is negative. Its mean value for the ten human males tested was -0.006. The linearity did not extend, however, beyond the limits of 1,500 on the one hand and 12,000 on the other, and while it was believed that the slopes calculated above were measures of the phagocytic power of the individual's blood, nevertheless it proved more convenient to judge such power by direct inspection of the plotted data rather than from calculation. Since the range of leucocytes is greater where dog's blood was used it becomes clear that the curve is not a straight line.

The results with the normal human blood are shown in Chart 1. Each individual case has been considered separately. The effect of changing the number of leucocytes is evident. The results from a study of the blood of the ten dogs have been put together in Chart 2. One notes here the straightening

out of the curve as the higher numbers of leucocytes are encountered. It also shows the general agreement between samples of blood from different dogs.

The following experiments were planned to meet the criticism of Mudd⁴ that the results obtained in an earlier report were due to the technic practiced. The suggestion was made that agitation and the employment of an "excess" of bacteria would obviate the "error." Just what is meant by "excess" is not clear. A suspension containing 800,000,000 living staphylococci per c.c. was used as suggested by Mudd and the same streptococcus suspension employed in earlier experiments. This was a heat-killed culture of a hemolytic streptococcus in saline suspension of about the same density as the staphylococcus suspension.

In the first experiment 0.3 c.c. of whole heparinized dog's blood was added to each of a series of tubes. Each sample of blood contained different numbers of leucocytes. Otherwise they were the same. To each tube was then added 0.1 c.c. of the streptococcus suspension. To a second series of tubes of the same blood was added a more concentrated suspension of the streptococcus. This was accomplished by centrifuging the original suspension and resuspending the sedimented bacteria in about half the amount of saline originally used. The tubes were then placed in a small rack and oscillated by hand at room temperature for ten minutes. Four rotations through an angle of approximately 160° were made per minute. The tubes were then placed in ice water, smears made and stained by Wright's method. One hundred polymorphonuclear leucocytes were counted in each smear.

EXPERIMENT I

NUMBER OF LEUCOCYTES*	PER CENT PHAGOCYTOSIS	
	DILUTE SUSP.	HEAVY SUSP.
8100	36	98
8100	44	100
5100	58	99
4300	51	100
3300	69	99

*Since whole heparinized blood was used the leucocyte counts could be made with one per cent acetic acid.

The test using a dilute suspension shows the effect of varying the number of leucocytes. With the concentrated suspension this effect is not apparent. This also shows the importance of using a properly prepared suspension.

In Experiment II one series of paraffin stoppered tubes containing 1 c.c. of heparinized whole dog's blood and 0.3 c.c. of a suspension of living *Staph. albus* (800,000,000 per c.c.) and a second series of tubes containing 1 c.c. of the blood and 0.3 c.c. of the killed streptococcus suspension were placed in a rotating machine making 16 rotations per minute. The machine was set in a 37° C.

EXPERIMENT II

NUMBER OF LEUCOCYTES	PER CENT PHAGOCYTOSIS	
	S. ALBUS	ST. HEMOLYTICUS
13200	89	53
10600	100	78
6100	99	99
4300	97	100

incubator and run for about ten minutes. The tubes were then placed in ice water, and smears made in the usual manner.

It is quite obvious that 0.3 cc of the staphylococcus suspension was too much. The results with the streptococcus suspension demonstrate the influence of the number of leucocytes.

Experiment III was planned to remedy the error due to the use of too many bacteria. Consequently 0.1 cc of the bacterial suspension was used with 1 cc of blood. The tubes were rotated at 37° C as in Experiment II except that smears were made both after five minutes and ten minutes incubation, the tubes being placed in ice water while smears were being made.

EXPERIMENT III

	NUMBER OF LEUCOCYTES	AVERAGE NUMBER OF <i>S. ALBUS</i> PER PHAGOCYTE	PER CENT PHAGOCYTOSIS <i>S. ALBUS</i> ST. HEMOLYTICUS
After 5 min inc	7200	2.4	46
	5500	1.5	45
	4100	2.4	49
	2800	2.4	65
After 10 min inc	7200	1.9	47
	5500	2.0	70
	4100	2.5	65
	2800	2.2	78

Although the variation in the number of leucocytes is not very great, a tendency toward higher percentages with fewer leucocytes is quite plain. This is somewhat more apparent after ten minutes' incubation than after five minutes'. Even the phagocytic index for *S. albus* is seen to increase as the number of leucocytes decreases.

It is evident from these findings that the number of leucocytes present in the phagocytic test is a significant factor even when "agitation" is used.

SUMMARY

The results of the experiments here reported show clearly the importance of a knowledge of the number of leucocytes engaged in phagocytic tests especially if samples of blood from different sources or from the same individual at different times are to be compared.

The relation of the leucocyte count to the percentage phagocytosis is brought out. By applying a simple formula one can compare the phagocytic power of an individual's blood at different times. When enough points are available the graphic method can be substituted for the mathematical.

CONCLUSIONS

Comparisons of percentage phagocytosis based on phagocytic mixtures containing unequal numbers of leucocytes are unreliable. A method for estimating the phagocytic power of the blood, which takes this into consideration, is presented.

REFERENCES

1. Dodd, H. B.: A Modification of Leishman's Method of Estimating the Opsonic Index, *Brit. M. J.* 2: 948, 1907.
2. Fleming, A.: Some Observations on the Opsonic Index, With Special References to the Accuracy of the Method and to Some of the Sources of Error, *Practitioner* 80: 607, 1908.
3. Hektoen, L.: Variations in the Phagocytic and Other Powers of Leukocytes, *J. A. M. A.* 57: 1579, 1911.
4. Jung, R.: Correlation Between Number of Leucocytes and Percentage Phagocytosis, *Proc. Soc. Exper. Biol. & Med.* 29: 981, 1932.
5. *Ibid.*:
6. Mudd, S., McCutcheon, M., and Lucke, B.: Phagocytosis, *Physiol. Rev.* 14: 210, 1934.

A ROUND TABLE FOR USE IN PATHOLOGIC HISTOLOGY*

J. McLEAN, M.D., NEW YORK, N. Y.

THIS table was designed to seat and provide comfortable working conditions for eight persons engaged in the examination of sections (Fig. 1). It was made circular in shape to secure freedom from elbow touching and an uninterrupted stream of light from a central source as illustrated. The supporting structure is hexagonal in form and contains twelve cabinets, necessarily somewhat tri-

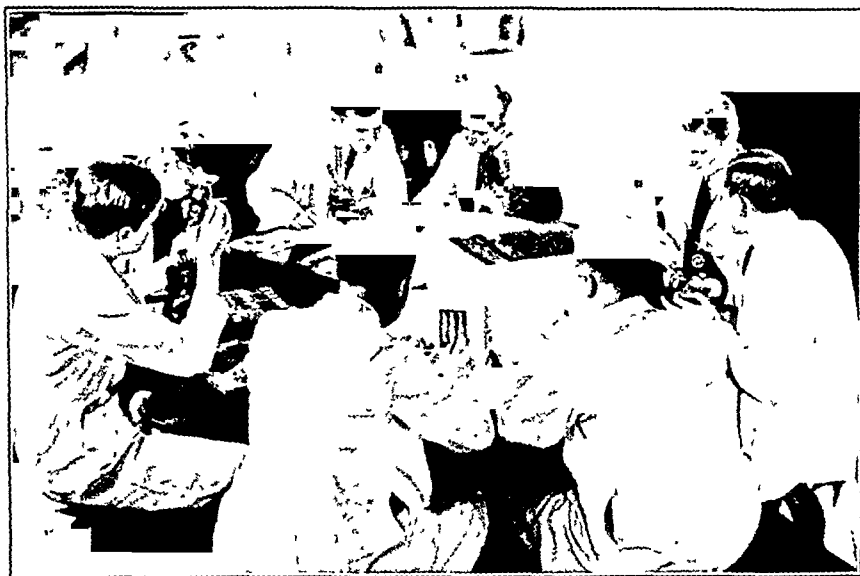


Fig. 1.—Eight persons can comfortably use the table. There is room for microscopes, trays of slides, reference books, etc.

angular in shape, which easily accommodate the largest microscope box, as well as a book or two and some accessories. The main top of the table is for the unobstructed use of the microscope, the writing of diagnoses and perhaps the consultation of a reference book. The trays containing the slides being examined

*From the Memorial Hospital
Received for publication, June 9, 1935.

54704

are on the smaller table top. These can be reached by all, or passed from one to another. The sides of this smaller table top provide recesses for reprints, ash receivers, etc.; as well as compartments of shelves for the storage of seven trays comparatively free from dust (Fig. 2).

The circular form of the table economizes space, but what is more important, it makes discussion easier and more direct. The round table is adapted to seminars in pathology, biology, or other branches of science employing the microscope.

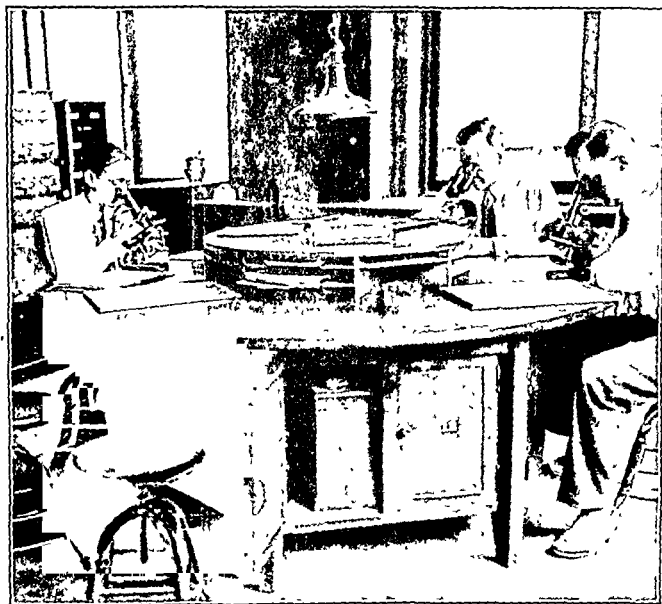


Fig. 2—Showing light (suspended by a chain), cabinet and top table detail.

The table was originally designed for the use of the eight Fellows and four internes of this institution, in order to provide reasonably safe storage for their microscopes and convenient facilities for the daily study of the current sections of the Department of Pathology.

However, formal postgraduate instruction in the Memorial Hospital, while confined to the above groups, is actually given informally to others. For instance, pathologists of several hospitals in the Metropolitan area attend the weekly clinical conference of the Medical Staff, at which interesting diagnostic or therapeutic problems or results are presented with the patients; and spend the afternoon in the pathology department studying the slides which have come through during the preceding week. Then, in addition, many pathologists spend

from a few weeks to a few months (especially in the summer months) studying the daily slides made up of biopsies, operative and autopsy material of this hospital, as well as many sections sent here from widespread sources for diagnosis. There is also available the Ewing Tumor Collection of 5,000 slides, easily accessible in a cabinet next to the table; and a Special Collection of sections selected from time to time from the daily material which illustrate certain pathologic conditions, or are sections difficult to classify exactly. Finally, as the

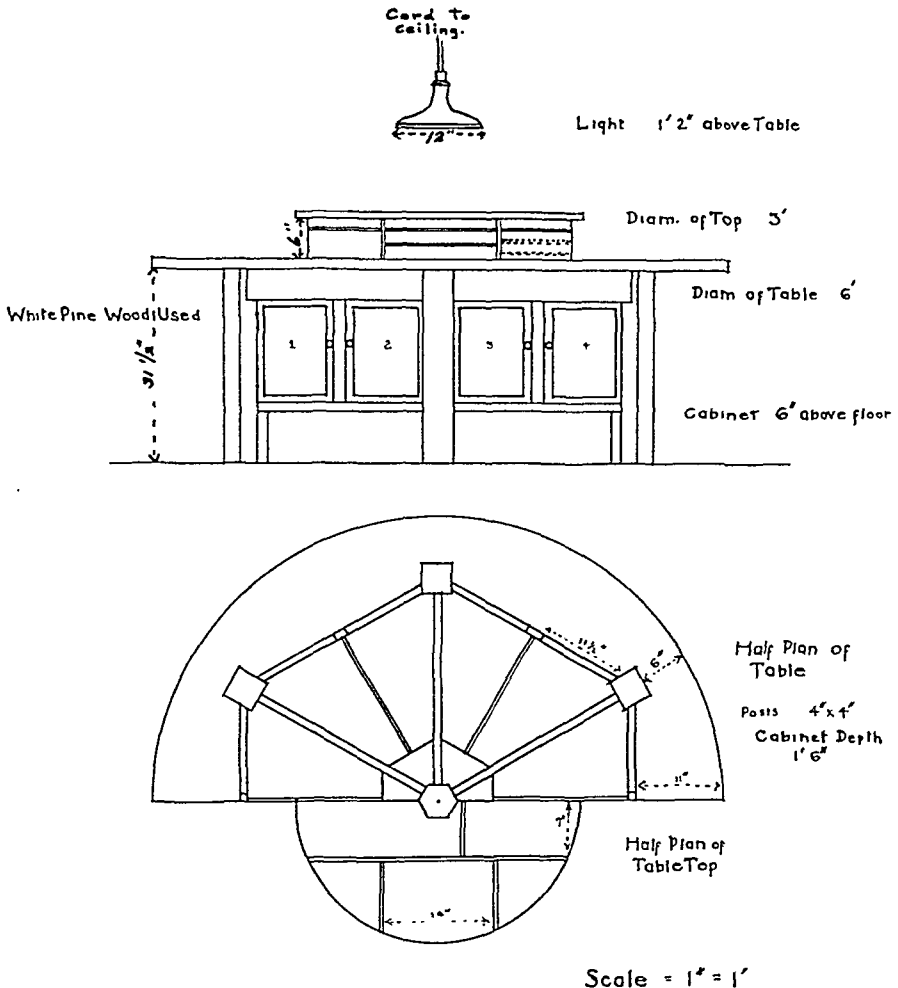


Fig. 3.—Plan and elevation of table.

round table is located in the Museum, there are 750 gross specimens plainly displayed, and for each there is a large envelope containing the sections thereof and the clinical history of the case, together with photographs and roentgenograms. As the Museum is next to the pathologic laboratory, the gross operative material can be conveniently examined as it comes from the operating rooms.

During the academic year the table is likewise of considerable convenience for under-graduate instruction. The Memorial Hospital offers the senior class

students of the Cornell University Medical College (an affiliate) a four weeks' systematic course in cancer. The students electing the course are received in groups of eight every month of the academic year.

The table has been in use over a year, and its use has not suggested any improvement in design.

Several of the pathologists from other laboratories who have studied here have expressed a desire to have such a table built at their institutions, and for this reason the plan and elevation of the table, photographs, and the following data are given.

The table was constructed in the carpenter shop of this hospital by a master carpenter regularly employed here. Due to the present economic conditions it had to be built as reasonably as possible, yet staunchly to make the expenditure an investment. The main top is of whitewood boards, 12 inches by $1\frac{1}{2}$ inches, and is six feet in diameter. The small top is of five ply wood and is three feet in diameter. The cabinets were made of white pine and each has a lock. Tops are painted black and waxed, other wood is stained a dark cherry color. The proper elevation of the main top, as well as the height of the lamp, was carefully considered. These are given in the drawing (Fig. 3). The lamp was furnished by the North Light Corporation, 30 West 15th Street, New York City. It contains a 200 watt Mazda lamp the light of which passes through a blue stained glass cut to diffuse the light. This arrangement and location of the light gives adequate illumination, equally to all working at the table.

Cost of the construction of the table follows:

Lumber	\$46 70
Hardware	20 00
Carpenter's wages	65 00
Printer's wages and material	6 50
	<hr/>
	\$138 20

DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILBUFFE, M.D., ABSTRACT EDITOR

FRUCTOSURIA, Essential, Silver, S., and Reiner, M. Arch. Int. Med. 54: 412, 1934.

Three cases of essential fructosuria are reported, and metabolic studies of two are presented.

This rare anomaly is viewed as a specific, probably inborn, error of metabolism localized primarily in the liver, where the authors believe, a specific enzymatic deficiency exists resulting in impaired ability to fix fructose as glycogen. The metabolism of other carbohydrates is undisturbed. The relative insignificance of insulin and epinephrine in the metabolism of fructose compared with dextrose is pointed out, and the possible intermediary metabolism of sorbitol is discussed.

HEMOGLOBIN, Bile Pigment and Regeneration of the Effect of Bile Pigment in Cases of Chronic Hypochromic Anemia, Patek, A. J., and Minot, G. R. Am. J. M. Sc. 188: 206, 1934.

Nine selected patients with chronic hypochromic anemia were studied to determine whether bile pigment could assist in hemoglobin production.

Concentrated bile pigment alone caused not a reticulocyte response but an increase of hemoglobin, about 7 per cent in ten days. This indicates that in certain anemic patients bile pigment can be absorbed from the gastrointestinal tract for building hemoglobin.

After a reticulocyte response occurred to a suboptimal dose of iron, bile pigment was fed directly with the same dose of iron, and there followed a second reticulocyte response. The second response was sometimes of greater magnitude than the first. This indicates that bile pigment in some unknown manner can facilitate either iron absorption or utilization.

One patient who could not obtain in fourteen months a normal hemoglobin level with large daily doses of iron promptly increased her hemoglobin concentration when bile pigment was fed in addition to iron.

It is suggested that in certain cases of hypochromic anemia there may be a deficiency of a useful material that is contained in bile pigment in addition to iron deficiency.

POLIOMYELITIS, an Enterodepressant Factor in the Stools and Spinal Cords of Monkeys Infected with, Toomey, J. A., and Von Oettingen, W. F. Am. J. Dis. Child. 48: 30, 1934.

Potent poliomyelitis virus suspensions in physiologic solution of sodium chloride acted as a depressant on the rabbit's intestine. The depression was probably local.

Serum from normal monkeys and from human beings in the acute stages of poliomyelitis did not prevent depression of the intestine, while convalescent human and monkey poliomyelitis serums did.

There was a toxic factor in the stools of monkeys with experimental poliomyelitis which was absent in those specimens obtained from normal monkeys.

None of the specimens of stools obtained from human beings with contagious diseases except influenza, contained a depressant factor.

LYMPHOGRANULOMA INGUINALE Cultivation of Virus of, Tamura, J T J A
M A 103 408 1934

The author believes that he has succeeded in cultivating the virus by the following method

When pus is removed aseptically from a bubo that has not been exposed to external contamination and is found to be bacteriologically sterile, it is diluted 1:5 with sterile saline solution. Now, when from 0.02 to 0.03 c.c. of the diluted pus is planted in the Tyrode's solution containing a piece of guinea pig kidney or liver and incubated at 37.5° C, a peculiar cloudiness appears throughout the supernatant fluid in from thirty six to forty eight hours. Control tubes of the medium incubated at the same time remain perfectly clear. This cloudiness can be transmitted from tube to tube and in one instance was carried through twenty four subcultures, when the procedure was discontinued.

When transfer is made from twelve day old cultures it is successful. In a fourteen day old culture the cloudiness seems to settle out and when the clear supernatant fluid is subcultured it no longer produces cloudiness.

LEPROSY, Mitsuda's Skin Reaction in Hayashi, F Internat J Leprosy 1 31 1934

Preparation of the vaccine Fresh nodules are boiled in physiologic salt solution for from thirty to sixty minutes, and ground in a mortar. To 1 gm. of the ground nodular material are added 20 c.c. of the salt solution used in the boiling, fresh salt solution being added, if necessary, to make up the required volume. The whole is filtered through gauze and the filtrate heated at 60° C for one hour. Carbolic acid is added to make 0.5 per cent concentration. One tenth cubic centimeter of the material prepared in this manner is used for an intracutaneous injection.

The reaction The reaction is looked for on the eighth, sixteenth, and twenty fourth days after the injection, as late reactions may not appear before the second or third week. In the negative reaction in nodular leprosy there is slight reddening which lasts only from two to four days, and never over eight days.

In reading the positive reactions, those with infiltrated areas 0.3 to 0.5 cm. in diameter are classed as one plus (+), those 0.5 to 1.0 centimeter as two plus (++), and those over 1.0 cm. and those with pus formation as three plus (+++). Some reactions start eruptively, while others begin more diffusively, the latter type with large areas gradually becoming strongly positive.

Results obtained Of the 192 lepers tested, 64 were neuromacular and 125 nodular cases. Of the former all but two (97 per cent) showed positive reactions, while of the nodular cases all but eleven (91 per cent) were negative.

Significance of the reaction The intracutaneous injection of leprosy bacilli produces no reaction in patients who have reached the nodular state, with unlimited proliferation of the bacilli.

The positive reaction appears only in normal individuals resistant to leprosy and in lepers in the neuromacular stage in which a certain degree of resistance is to be presumed. In no case does the negative reaction appear in the normal, tuberculous, or syphilitic.

LYMPHOGRANULOMA INGUINALE Burney L E Ven Dis Inf 15 233, 1934

Lymphogranuloma inguinale is a specific disease of venereal origin.

Definite proof that the etiologic agent is a filtrable virus has been established.

The chief symptoms and clinical findings are an evanescent primary lesion, not always found, inguinal and iliac lymphadenitis usually with numerous fistulas running a chronic course of several months to several years, and symptoms of a mild septicemia during the first stage of the lymphadenitis.

In women, because of anatomical difference in lymph drainage, the infection tends to localize in the lymph plexuses around the rectum producing scar tissue and ultimate stricture.

The intradermal test developed by Frei is specific for lymphogranuloma inguinale and from various observations apparently remains positive for many years and probably for life.

Treatment consists mainly of partial extirpation of affected nodes in conjunction with drainage and intravenous use of antimony and potassium tartrate. For those cases presenting rectal structure, surgery is the only recourse.

The disease is not confined to the tropics or even to seaports, but must be looked for in inland cities as well. Of the 4 cases reported, 3 were from Arkansas and 1 from Mississippi.

HODGKIN'S DISEASE, Etiology of: Skin Reaction to Avian and Human Tuberculin Proteins, Steiner, P. E. Arch. Int. Med. 54: 11, 1934.

Tuberculin protein (Seibert) was used in performing intracutaneous skin tests on thirty-five patients with Hodgkin's disease (lymphogranulomatosis) and on thirty-eight controls with a variety of lymphoma. Tuberculin proteins prepared from both avian and human strains were used in comparative tests for the purpose of obtaining information on the possible etiologic rôle of the avian tubercle bacillus in Hodgkin's disease.

No evidence of specific sensitization to the avian tuberculin protein was obtained in these tests. A marked desensitization (or absence of sensitization) was found to both proteins in the cases of Hodgkin's disease. This relative absence of "normal adult sensitization" was of diagnostic value, especially in the differential diagnosis of tuberculous adenitis. The incidence of absence of sensitization was greater in the group with Hodgkin's disease than in the groups of other lymphomas, which admittedly were small.

The interpretation was made either that (1) the process of Hodgkin's disease desensitizes its victims to these tuberculin proteins or that (2) Hodgkin's disease usually occurs in persons in whom development of the normal sensitization to the tuberculin protein is impossible.

It is difficult to conceive of either of these phenomena as occurring in a disease absolutely unrelated to tuberculosis.

BLOOD, in Healthy Young Infants, Washburn, A. H. Am. J. Dis. Child. 50: 413, 1935.

The results of 908 total leucocyte counts on twenty healthy infants together with an analysis of 608 differential counts on seven of these infants are presented by means of tables and charts.

The majority (800) of these counts were done between the hours of 9:00 A.M. and 5:00 P.M., the remainder being done during the night. In most instances 8 counts were made in one day, from six to twelve such series being done on each baby during the first three to six months of life.

Concerning the leucocyte counts of the blood of healthy infants from the second to the twenty-sixth week of life the following conclusions seem justified:

The total leucocyte count may vary from 5,000 to 24,000 without indicating the presence of demonstrable disease, but the majority of the counts (the middle 80 per cent) lie between 8,000 and 16,500.

Fluctuations in the counts of all the white blood cells during the hours of the day and night show no tendency to orderly rhythm, vary a great deal from one day to another and show no correlation with age, activity, feedings, or the routine events of the day.

The curve of the lymphocyte counts is the only one which shows a persistent tendency to parallel that of the total leucocyte counts.

The limits of variation which may be expected in each of the main groups of leucocytes are approximately as follows: lymphocytes, from 2,300 to 15,700 per c.mm. (from 27 to 88 per cent); polymorphonuclear neutrophils, from 500 to 9,500 (from 5 to 63 per cent); all the granulocytes, from 650 to 11,500 (from 10 to 68 per cent); and monocytes, from 150 to 2,400 (from 1 to 18 per cent).

The variation in the differential leucocyte counts of individual infants or in any one infant from time to time is as striking as that previously reported in the total leucocyte counts

BLOOD, in Healthy Young Infants Washburn A H Am J Dis Child 50 395, 1935

The routine technic and classification used in differential white blood cell counts have been compared with more specialized methods

The first study consisted in comparing 100 differential counts on fixed smears prepared with Wright's stain with the same number of counts performed on fresh drop preparations supravitality stained

Charts are presented which show the variation in the percentage of each cell type in comparing (1) differential counts on preparations stained with the vital stain and with Wright's stain, (2) a second series of counts on preparations stained with Wright's stain and (3) the average of these two counts on preparations stained with Wright's stain with the counts on preparations prepared with the vital stain

There was no constant or striking tendency for any one cell type to appear more frequently in the fresh drop preparation than in the fixed smear or vice versa

The deviations noted in each of the three sets of comparisons were greater than those usually given, being smallest for the comparison of the first and second series of counts on the fixed smears prepared with the Wright stain

From the study it was concluded that a fixed smear stained with a reliable Wright's stain may show the same distribution of white blood cells in infant's blood as is shown in a fresh drop stained with neutral red and janus green B

The second study consisted in a critical analysis of the Arneeth Schilling and similar classifications of the granulocytes

Tables are given to show the variations which were found in a small group of healthy infants when the various methods were used

The uncertainty of classification as well as the time consuming nature of the procedure are felt to overbalance any value gained by grouping the neutrophils according to the number of nuclear lobes

By Schilling's method the lack of constancy and reliability in classification as well as the amount of variation found are much greater for the stab cell than for the more definitely immature juvenile form or myelocyte

It was concluded that in the young infant the simplest, safest and most significant classification of the granulocytes is accomplished by dividing them into groups of myelocytes immature polymorphonuclears polymorphonuclears eosinophiles and basophiles

For routine work and for most practical purposes in clinical medicine the routine method of staining and counting the white blood cells seems to be as satisfactory as and safer than, the special methods studied. This is based on the assumption that the smear is carefully made the Wright stain is of good quality and the count is done thoroughly and honestly

SYPHILIS, Congenital, Diagnosis by Means of Dark Field Examination of Scrapings from Umbilical Vein Ingraham N R J A M A 105 560, 1935

In this series of cases dark field examination of the umbilical vein has been instrumental in detecting syphilis in 19 (21.8 per cent) of the 87 living offspring of syphilitic mothers on whom it was tried with an accuracy which might be considered 100 per cent and at a time in the infant's life when most other clinical criteria and laboratory aids are unsuccessful. Since 75 of the living infants studied however, were actually proved by observation to have syphilis, it is needless to say that a negative dark field examination does not rule out syphilis in the child but merely indicates the necessity of studying the infant by other methods. The fact that in 54 per cent of the diseased children the diagnosis was made within a few hours after birth seems to indicate that this method has a diagnostic value which somewhat overshadows its cumbersome nature. It is truly impractical to examine a specimen of the cord obtained at every delivery in a large maternity service,

but when the diagnosis of syphilis is made in the mother, as it should be, before delivery takes place, then the suspected syphilitic specimens can be examined without placing too great a burden on the physicians in charge, and the diagnosis of congenital syphilis can be established immediately in a limited number of cases.

TUBERCULIN TESTS, Comparison of, Anzen, G. *Am. J. Dis. Child.* 50: 104, 1935.

In the present investigation the plaster and the Pirquet test yielded consistent results in 98 per cent of the cases. In 0.5 per cent of the cases the plaster test gave a positive reaction while the reaction to the Pirquet test was entirely negative. In febrile states the plaster test proved much more sensitive than the Pirquet test. Even when both tests gave positive results the plaster test, in the majority of cases, yielded the more powerful reaction. Were the plaster test alone employed, only 1.25 per cent of the positive reactions would fail to be detected.

BACTERIOLOGY: Simple Method of Supplying Carbon Dioxide in Jars for Bacteriologic Cultures, Thompson, L. *Am. J. Clin. Path.* 5: 313, 1935.

The method to be described is very simple and requires no special apparatus. The carbon dioxide is derived from sodium bicarbonate which has been mixed with sulphuric acid. Two solutions are prepared: one, a molar solution of sodium bicarbonate, which is made by dissolving 84 gm. of sodium bicarbonate in water and making the volume up to one liter; the other, a solution of sulphuric acid, which is made by diluting concentrated sulphuric acid so that 1 c.c. of acid is added to 29 c.c. of water. The acid so made is sufficient to react with the bicarbonate solution part for part and still leave a slight excess of acid. Sulphuric acid, being nonvolatile, does not affect the cultures. Each cubic centimeter of the bicarbonate solution should furnish 22.4 c.c. of carbon dioxide.

An ordinary 3 quart museum jar has proved very satisfactory. A rubber ring is used for a seal, but no other precautions have to be taken to prevent loss of carbon dioxide from the jar. The plates that are to be incubated are placed in the jar; then, a sufficient quantity of the bicarbonate solution to produce approximately a 10 per cent concentration of carbon dioxide within the jar is placed in a small wide-mouth bottle. The cover is put in place and an amount of the sulphuric acid solution equal to the amount of bicarbonate solution that is employed is added with a pipette, through a hole in the cover. The stopper is restored to the hole as promptly as possible, and the jar is placed in the incubator.

Erratum

On pages 651 and 652 in the March issue, article by Spohr and Landy, the cuts are transposed over the legends. The cut appearing on page 651 should appear on page 652 over the legend for Fig. 2.

The Journal of Laboratory and Clinical Medicine

VOL 21

MAY, 1936

No 8

CLINICAL AND EXPERIMENTAL

BACTERIOLOGIC STUDIES OF THE URINE, UTILIZING A SELECTIVE METHOD OF CULTURING*

MYER SOLIS COHEN, A B M D, PHILADELPHIA, PA

BACTERIOLOGIC studies of the urine are attended with considerable uncertainty and confusion. A common error is to regard as of etiologic significance the bacteria that grow in a culture of the urine or that are seen in the stained smear. Yet it is known that the mere presence of a germ on a mucous membrane or on the secretion from it does not spell infection, and that in a subacute or chronic infection the predominance of a microbe in a culture does not necessarily indicate any causal relationship between it and such infection. This latter fact is well brought out by my study of 384 cultures in chronic and focal infections, made in glucose brain broth. The organism that predominated in 169 (44 per cent) of these cultures failed to grow in the patient's whole, coagulable blood, thus probably indicating its lack of pathogenicity for him.

The urologist, who is anxious to know what organism is causing an infection, wants the bacteriologist to state not only what organisms are present but which of these are of etiologic significance and which may be ignored as not being pathogenic for the particular patient, however pathogenic they may be for other human beings or for animals. He also is interested in their source or origin.

METHODS FOR DETERMINING THE INFECTIVITY OF BACTERIA FOR THEIR HOST

Some bacteriologists employ various serologic tests to determine infectivity, although, according to Topley and Wilson,¹ the antibody titer of a particular sample of serum, as measured *in vitro*, is not highly correlated with its power to protect *in vivo*. These authors regard the lack of correspondence between the

*Received for publication July 1 1935

presence of precipitins and agglutinins on the one hand, and the clearing capacity on the other, as obvious. They also state that resistance to a particular bacterium has frequently been associated with the entire absence of demonstrable complement-fixing bodies.

Intracutaneous testing is employed by many, including Freiberg and Dorst,² Cox,³ and Eiman,⁴ but has proved disappointing as a diagnostic measure in the hands of Swift,⁵ Gay,⁶ Mackenzie and Hanger,⁷ Bürgers and Wolffheim,⁸ Moore,⁹ Short, Dienes and Bauer,¹⁰ and others. Kolmer and Boerner¹¹ say its exact value or status is as yet unknown. I¹² have shown that it is questionable whether in a mixed culture intracutaneous testing can identify the bacteria which are infecting the patient and that it probably is unreliable for selecting bacteria in the preparation of vaccines.

Rosenow's¹³ theory of selective affinity is not regarded as valid by most workers and in the opinion of Topley and Wilson¹ has not yet been placed upon a firm foundation.

Heckel, Jensen and Wood¹⁴ describe a new cataphoresis technic for identifying and isolating the causative bacteria in "nonspecific" genitourinary tract infections, by means of which they claim to have been able to isolate and determine the infecting microbes, usually streptococci, in a given case of "nonspecific" genitourinary tract infection. Their announcement, however, met with considerable criticism (Wessen,¹⁵ Herrold¹⁶), and until their method has received sufficient trial and their results with it sufficient corroboration, it would be advisable to postpone judgment as to the value of the method.

Inasmuch as, with one exception, the various selective tests are applied only to organisms that have grown upon culture media, they would be valueless in from 11 to 18 per cent of all cultures. For the infecting organism failed to grow up in 11 per cent of Boerner and my¹⁷ 404 cultures in deep tubes of glucose brain broth and in 18 per cent of 600 cultures made by Lowe.¹⁸

THE PATHOGEN-SELECTIVE CULTURE

The one exception is the pathogen-selective culture,^{17, 19-22} which, in chronic and focal infections, utilizes the bactericidal power of the patient's fresh, whole, coagulable blood for selecting the etiologically important organisms from a mixed culture. Wright and his associates²³ have pointed out that the whole blood of an animal is often bactericidal, when the separated serum or plasma shows no such effect. With Heist and S. Solis-Cohen, I²⁴ demonstrated that, while the serum of the resistant pigeon did not show apparent difference in its action from that of the susceptible mouse, or rabbit, the whole blood of the former was actively bactericidal for the pneumococcus, while that of the mouse, or rabbit, showed no such action. These observations were confirmed by Bull and Bartual,²⁵ Smiley,²⁶ and Robertson and Sia,²⁷ while Matsunami and Kolmer²⁸ found a similar parallel between the varying susceptibility of laboratory animals to meningococcic infection and the bactericidal action of their blood in vitro. Using the Heist and Solis-Cohen technic on rats of three categories, those highly susceptible to plague, those highly immune, and those possessing various grades of immunity, Malone, Avari and Naidu²⁹ showed that a definite correlation exists between the bactericidal power of the blood of rats and their immunity to plague.

Topley and Wilson¹ regard the association between the growth inhibitory action of the whole blood and the clearing capacity as very close. Heist, S Solis Cohen and I³⁰ showed that human susceptibility and resistance to meningococci could also be demonstrated and measured *in vitro* by the whole blood test. Heist and I²¹ cultured various organisms, present in different infections, in the fresh, whole, coagulable blood of their host and of one or more human controls *in vitro*, as well as in defibrinated blood, and found that the bloods of different individuals differed in the degree of their bactericidal power against the same organism.

Favorable results with the use of this culture method have been reported by Beckley,³¹ Crowe,³² Graham,³³ Lowe,^{18, 34} Shearman,³⁵ Thomson and Hill,³⁶ Walls,³⁷ Ramsey and Pearce,³⁸ and Barlow.³⁹ A detailed description of the technic is given in Kolmer and Boerner's *Approved Laboratory Technic*.¹¹

In the pathogen selective method of culture the material to be cultured is inoculated simultaneously into a rich culture medium, such as glucose brain broth or hormone broth, and also in the patient's fresh, whole, coagulable blood *in vitro*. After a primary incubation of twenty four hours, both cultures are examined and the organisms present in each are studied for identification. In subacute, chronic and focal infections, the organisms capable of growing in the fresh, whole, coagulable blood of the patient are regarded as those which are most pathogenic for the individual. One of the advantages of the method, therefore, is that in such cases it furnishes a means for selecting the etiologically important organisms from a mixed culture. An analysis of 404 pathogen selective cultures in chronic and focal cases made by Boerner and myself¹⁷ shows that organisms usually classified as pathogenic for man were the ones that grew most frequently in the patient's whole, coagulable blood—namely, streptococci and staphylococci. Of the organisms most frequently found, the percentages of each that grew in the patient's blood were as follows: Streptococci (hemolytic) 78.1, *Staphylococcus aureus* 72.5, streptococci (viridans group) 65.0, streptococci (non hemolytic) 61.2, *Corynebacterium pseudodiphthericum* 42.8, *Diplococcus pneumoniae* 40.9, *Staphylococcus albus* 35.1, gram negative cocci (unidentified) 21.0, gram negative bacilli (unidentified) 9.5, *Neisseria catarrhalis* 0, and *Neisseria sicca* 0.

BACTERIOLOGIC STUDY OF URINE CULTURED BOTH IN A RICH CULTURE MEDIUM AND IN THE PATIENT'S WHOLE, COAGULABLE BLOOD

Fifty-six specimens of urine from 44 patients have been cultured both in a rich culture medium and in the patient's fresh, whole, coagulable blood. In 4 cases the earlier capillary tube (Heist-Lacy) method²⁴ was employed and in the remainder the pathogen selective (test tube) method (referred to by Beckley,³¹ and Kolmer⁴⁰ as the Cohen-Heist method). Two of these cases were reported in 1920⁴¹ and some of the others at various times since.^{19, 21}

In making a pathogen selective culture of the urine I introduce into the urine an ordinary cotton applicator which is then introduced into a tube of enriched medium, such as glucose brain broth or hormone broth. Another applicator, after being introduced into the urine, is rubbed on the bottom and sides of a sterile empty test tube, into which is then placed 3 to 5 c.c. of the patient's fresh, whole, untreated blood, obtained from a vein—not blood serum.

or heparinized or citrated blood or defibrinated blood, as employed by Boerner and Mudd,⁴² Flemming,⁴³ Hare,⁴⁴ and Mackie and his associates.⁴⁵ Both tubes are incubated for twenty-four hours and then are plated, after which the organisms that grow up are identified. I have not employed the refinements of technic suggested by Lowe¹⁸ and Shearman,³⁵ although they probably make for greater accuracy.

Lowe¹⁸ stresses the importance of ensuring equality of distribution of inoculum between the two cultures, so that the results may be reasonably comparable, believing it obvious that the implantation of infected material into the patient's blood specimens should not be unduly heavy. He therefore, as far as possible, has standardized the emulsion of urinary deposit to be not greater than a strength approximately similar to a bacterial emulsion of 2,000 million organisms per c.c., from which three loops are inoculated into 5 c.c. of blood.

Shearman³⁵ likewise emphasizes that the amount of inoculum used must be very small, because the blood varies in its bactericidal powers and because specimens used as the inoculum vary considerably in their bacterial content. For this reason he has found it advisable when making the cultures to use two separate amounts, each of 5 c.c. of the patient's blood, and to vary the amount of inoculum used in each. In one 3 loops of an emulsion made from the specimen to be examined and of the density described by Lowe¹⁸ are used as the amount of inoculum and in the other only one loop is used. In practice he does not always get the same cultural result from each tube, as it not infrequently happens that inhibition is prevented in the tube containing the larger amount of inoculum, either because of the exceptionally high bacterial content of the inoculum or because of a low bactericidal action of the patient's blood, so that a mixed culture results. In such cases the tube with the small amount of inoculum will usually yield a pure culture of the pathogenic organisms if such be present in the focus under examination.

In 5 of the 9 specimens of urine from my patients without urinary infection, the same organisms were present in the enriched broth medium and in the patient's blood, in 3 the germs present in the broth culture failed to grow in the patient's blood, and in 2 the bacteria that grew up in the two cultures were different (Table I).

Of the 31 urine cultures in cases with pyuria there were no microbes in either medium in 10 per cent, the same bacteria grew up in both media in 64

TABLE I

SIMULTANEOUS CULTURES OF 56 URINES BOTH IN A RICH CULTURE MEDIUM AND IN THE PATIENT'S WHOLE COAGULABLE BLOOD

	CASES WITH NO URINARY INFECTION	CASES WITH URINARY INFECTION		
		WITH PYURIA	WITHOUT PYURIA	TOTAL
Number of urines	10	31	15	46
Number contaminated	1	0	2	2
Number remaining	9	31	13	44
No germs in either culture	0	3 (9.7%)	4 (30.8%)	7 (15.9%)
Same germs in both cultures	5 (55.6%)	20 (64.5%)	6 (46.1%)	26 (59.1%)
Germs only in culture medium	3 (33.3%)	6 (19.3%)	3 (23.1%)	9 (20.5%)
Different germs in both cultures	1 (11.1%)	2 (6.5%)	0	2 (4.5%)

per cent, the organisms present in the broth failed to grow up in the blood in 19 per cent, while different germs grew up in the two media in 7 per cent (Table I)

Of the 13 urine specimens from cases of urinary infection without pyuria, no microorganisms were present in 31 per cent, the same germs were present in both media in 46 per cent, and the bacteria present in the broth failed to grow in the blood in 23 per cent, while in none were there different organisms in the two media (Table I)

When the three groups are combined, it will be noted that microorganisms grew up in 46 of the 53 pairs of urine cultures examined. In one fourth of these all the germs that grew up in the enriched broth were killed by the patient's blood, indicating the presence in that blood of a bactericidal power against them. In the other three fourths organisms grew up in the patient's blood, signifying the absence in that blood of any bactericidal power against them. While the cases are too few for generalizations, it would seem that only in three fourths of urines containing bacteria can the latter be said to be infecting the patient. Eight and eight tenths per cent of the total 34 cultures in the patient's blood contained organisms that failed to grow up in the enriched broth culture and consequently would have been missed entirely had no cultures been made in the patient's blood (Table I)

Colon bacilli constituted 14.3 per cent of the organisms found in the urines in cases with no urinary infection and 18.2 per cent in cases of urinary infection without pyuria, all being killed by the patient's blood. They constituted 37 per cent of the germs present in the urines in cases of pyuria, 65 per cent of them growing in the patient's blood (Tables II and III)

Lowe³⁴ reported that out of 20 cases showing bacilluria in carefully collected catheter specimens, in 14, or 70 per cent the organisms present were non pathogenic to the patient, which he regards as quite in keeping with clinical experience

Streptococci constituted 14.3 per cent of the bacteria found in the urine in my cases with no urinary infection and 9.1 per cent in cases of urinary infection without pyuria, all of them growing in the patient's blood. They constituted 10.9 per cent of the microbes found present in the urines in cases of pyuria, 80 per cent of them growing in the patient's blood (Tables II and III)

Staphylococci constituted 64.3 per cent of the microorganisms present in the urines in cases without urinary infection, two thirds of them growing in the patient's blood, 72.7 per cent in cases of urinary infection without pus, three fourths of them growing in the patient's blood, and 37 per cent in cases of pyuria, half of them growing in the patient's blood (Tables II and III)

Grouping the cases into those with pus and those without pus in the urine, colon bacilli constituted 16 per cent of the germs present in the urines from the cases without pyuria, and when present were of little etiologic significance. In cases of pyuria they constituted 37 per cent of the organisms found in the urines, two thirds of them being pathogenic for the patient and probably the causal organisms (Table IV). This would seem to partially confirm Cabot and Crabtree's⁴⁰ observation that cecal organisms are the etiologic factors in urinary infections unaccompanied by pus in the urine

TABLE II
BACTERIOLOGY OF URINE WITH REGARD TO PATHOGENICITY FOR PATIENT

ORGANISM	CASES WITHOUT URINARY INFECTION						CASES WITH URINARY INFECTION					
	CASES WITHOUT URINARY INFECTION			CASES WITH PYURIA			CASES WITH URINARY INFECTION			CASES WITHOUT PYURIA		
	FOUND IN URINE	GROWING IN BLOOD	NOT GROWING IN BLOOD	GROWING ONLY IN BLOOD	FOUND IN URINE	GROWING IN BLOOD	NOT GROWING IN BLOOD	GROWING ONLY IN BLOOD	FOUND IN URINE	GROWING IN BLOOD	NOT GROWING IN BLOOD	GROWING ONLY IN BLOOD
<i>Strep. hemol.</i>	1	1 (100%)	0	1 (100%)	1	1 (100%)	0	1 (100%)	1	1 (100%)	0	0
<i>Strep. vir.</i>	1	1 (100%)	0	0	4	3 (75%)	1 (25%)	2 (50%)	1	1 (100%)	0	1 (100%)
<i>Strep. nonhemol.</i>	3	3 (100%)	0	0	3	3 (100%)	0	1 (3.33%)	7	5 (71.4%)	2 (28.6%)	0
<i>Staph. aureus</i>	5	3 (60%)	2 (40%)	1 (20%)	13	4 (30.8%)	9 (69.2%)	0	1	1 (100%)	0	0
<i>Staph. albus</i>					1	1 (100%)	0	0				
<i>Staph. citreus</i>	1	0	1 (100%)	0	1	1 (100%)	0	1 (100%)				
<i>Staph. unidentified.</i>					1	1 (100%)	0	0				
<i>Pneumococci</i>					1	1 (100%)	0	0				
Gram-pos. cocci					1	1 (100%)	0	0				
Diphtheroids					3	2 (66.6%)	1 (33.3%)	0				
<i>B. coli</i>	2	0	2 (100%)	0	17	11 (64.7%)	6 (35.3%)	0	2	0	2 (100%)	0
<i>Bac. aerogenes</i>	1	0	1 (100%)	0	2	1 (50%)	1 (50%)	0				
<i>Bac. pyocyaneus</i>												
Total	14	8 (57.2%)	6 (42.8%)	2 (14.3%)	46	28 (60.9%)	18 (39.1%)	5 (10.9%)	11	7 (63.6%)	4 (36.4%)	1 (19%)

TABLE III

PRINCIPAL ORGANISMS FOUND, WITH ALL STREPTOCOCCI AND STAPHYLOCOCCI GROUPED TOGETHER CASES DIVIDED INTO THOSE WITH AND THOSE WITHOUT URINARY INFECTION

ORGANISM	CASES WITHOUT URINARY INFECTION						CASES WITH URINARY INFECTION					
	CASES WITHOUT URINARY INFECTION			CASES WITH PYURIA			CASES WITH PYURIA			CASES WITHOUT PYURIA		
	FOUND IN URINE	GROWING IN BLOOD	FAILING TO GROW IN BLOOD	FOUND IN URINE	GROWING IN BLOOD	FAILING TO GROW IN BLOOD	FOUND IN URINE	GROWING IN BLOOD	FAILING TO GROW IN BLOOD	FOUND IN URINE	GROWING IN BLOOD	FAILING TO GROW IN BLOOD
Streptococci	2 (14.3%)*	2 (100%)	0	1 (50%)	5 (10.9%)*	4 (80%)	1 (20%)	3 (60%)	1 (100%)*	1 (9.1%)*	1 (100%)*	0
Staphylococci	9 (64.3%)*	6 (66.6%)	3 (33.3%)	1 (11%)	17 (37.0%)*	8 (17%)	9 (53%)	1 (6%)	8 (72.7%)*	6 (75%)*	2 (25%)	1 (12.5%)
Bacillus coli	2 (14.3%)*	0	2 (100%)	0	17 (37.0%)*	11 (65%)*	6 (35%)	0	2 (18.2%)*	0	2 (100%)	0
Diphtheroids	0	0	0	0	3 (6.5%)*	2 (66.6%)*	1 (33.3%)*	0	0	0	0	0

*Percentage of all the organisms found in all the cases in the group

TABLE IV

PRINCIPAL ORGANISMS FOUND, WITH ALL STREPTOCOCCI AND STAPHYLOCOCCI GROUPED TOGETHER CASES DIVIDED INTO THOSE WITH AND THOSE WITHOUT PUS IN THE URINE

ORGANISM	CASES WITHOUT PUS IN URINE					CASES WITH PUS IN URINE				
	CASES WITHOUT PUS IN URINE		CASES WITH PUS IN URINE		FAILING TO GROW IN BLOOD	CASES WITHOUT PUS IN URINE		CASES WITH PUS IN URINE		FAILING TO GROW IN BLOOD
	FOUND IN URINE	GROWING IN BLOOD	FAILING TO GROW IN BLOOD	GROWING ONLY IN BLOOD		FOUND IN URINE	GROWING IN BLOOD	FAILING TO GROW IN BLOOD	GROWING ONLY IN BLOOD	
Streptococci	3 (12%)*	1 (100%)	0	1 (33.3%)	5 (17.8%)*	4 (80%)	1 (20%)*	9 (53%)*	3 (60%)	1 (6%)
Staphylococci	17 (68%)*	12 (70.6%)	5 (29.4%)	2 (11.8%)	17 (60.7%)*	8 (47%)	11 (65%)*	6 (35%)*	1 (6%)	0
Bacillus coli	4 (23.5%)*	0	4 (100%)	0	3 (6.5%)*	2 (16.6%)*	1 (8.3%)*	1 (8.3%)*	0	0
Diphtheroids	0	0	0	0	0	0	0	0	0	0

*Percentage of all the organisms found in all the cases in the group

Staphylococci constituted 68 per cent of the bacteria present in the urines without pyuria, 70.6 per cent of them being pathogenic for the patient and of etiologic significance. They constituted 37 per cent of the microbes found in the urines in cases of pyuria, half of them being pathogenic for the patient and of etiologic significance (Table IV).

Streptococci constituted 12 per cent of the organisms present in the urines in cases of urinary and other infections without pyuria, all of them being pathogenic for the patient and probably of etiologic importance. They constituted 10.9 per cent of the germs found in the urines with pus, 80 per cent of them being pathogenic for the patient and hence of etiologic importance (Table IV).

ADVANTAGES OF PATHOGEN-SELECTIVE CULTURES OF THE URINE

Pathogen-selective cultures of the urine would therefore seem to be more reliable and more informative than ordinary cultures, including those in glucose brain broth. In chronic and focal infections they may supply the need expressed by Wood⁴⁷ for real tangible evidence that can be applied as a useful aid in determining when bacteria are virulent for the particular individual, and may help to decide Horder's⁴⁸ query whether a germ present is the causative factor in an illness, and the question Young and Davis⁴⁹ regard as so difficult, whether the presence of streptococci denotes an additional danger to the patient. It may also differentiate mixed infections from secondary invasions and may aid in finding the elusive cocci, regarded by many as the causal factors.

The cases in which only the colon bacillus was present in the enriched broth, while a different organism grew in the patient's blood, may furnish the proof demanded by Chown⁵⁰ that colon bacilli may overgrow other pathogenic organisms.

CONCLUSIONS

1. Bacteriologic studies of the urine are attended with uncertainty and confusion.

2. The ordinary culture of the urine gives no information as to the infectivity for the host of the organisms that grow up.

3. The various serologic tests, intracutaneous tests, tests for selective affinity, and tests for cataphoresis are all of questionable value in determining the infectivity of bacteria for their host.

4. The pathogen-selective culture of the urine, in chronic and focal infections, utilizes the bactericidal power of the patient's fresh, whole, coagulable blood for selecting the etiologically important organisms from a mixed culture.

5. It reveals the presence of more types of organism than does glucose brain broth.

6. It would seem to show that only in three-fourths of urines containing bacteria can the latter be said to be infecting the patient.

7. It would seem to show that colon bacilli constitute about one-sixth of the organisms present in the urines in cases of urinary and other infections without pyuria, usually having little etiologic significance, and about one-third of the organisms present in the urines containing pus, two-thirds of them being of etiologic importance.

8 It would seem to show that staphylococci constitute over two thirds of the bacteria present in the urines in cases of urinary and other infections with out pyuria and about one third in the urines containing pus, nearly three-fourths of the former and half of the latter being of etiologic importance

9 It would seem to show that streptococci constitute 12 per cent of the germs present in the urines in cases of urinary and other infections without pyuria, all being of etiologic importance, and 10.9 per cent in the urines containing pus, 80 per cent having etiologic significance

REFERENCES

- 1 Topley, W W C, and Wilson, G S The Principles of Bacteriology and Immunity, London, 1929, Edward Arnold and Co, Vol II
- 2 Freiberg, Jos A and Dorst Stanley E The Allergic Joint, J LAB & CLIN MED 15 1109 1920
- 3 Cox, W C Autogenous Vaccines in Treatment of Chronic Sinus Infections and Nasal Allergy, Military Surgeon 73 121 1923
- 4 Eiman, John Discussion on Vaccine Therapy, Atlantic Med J 31 211, 1927
- 5 Swift, Homer F Rheumatic Fever Cecil's A Text Book of Medicine, ed 2, Philadelphia 1920 W B Saunders Co p 89
- 6 Gray, Leslie N Methods and Laboratory Technique Used in the Diagnosis of Allergic Diseases The Practitioner's Library of Med and Surg, New York, 1932, D Appleton and Co Vol II p 301
- 7 Mackenzie, George M, and Hinger Franklin M Jr Study of Hypersensitiveness to Derivatives of Hemolytic and Non Hemolytic Streptococci (Preliminary Report), Proc Soc Exper Biol & Med 21 442 1923 24
- 8 Burgers, Th J, and Wolfheim, W Zum Tonsillenproblem, Klin Wchnschr 10 1064, 1931
- 9 Moore, Merle W The Value of Skin Testing as an Aid in the Diagnosis of Allergic Diseases, Northwest Med 32 224, 1933
- 10 Short, Charles L, Dienes L and Bauer Walter Autogenous Vaccines in Rheumatoid Arthritis A Clinical Study and Critique Am J M Sc 187 615, 1934
- 11 Kolmer, John A, and Boerner, Fred Approved Laboratory Technique, New York, 1931, D Appleton & Co p 395
- 12 Solis Cohen, Myer A Comparison of the Relative Values of the Intracutaneous Skin Test and of the Pathogen Selective Culture in Selecting Bacteria for Vaccine From Mixed Cultures, Am J Clin Path 3 303 1933
- 13 Rosenow, Edward C Serologic Specificity of Streptococci Having Elective Localizing Power as Isolated in Various Diseases of Man, J Infect Dis 45 331, 1929
- 14 Heckel, N J Jensen, L B, and Wood I H A Study of Dissociating Streptococci and Their Electrical Charges in Infections of the Genito Urinary Tract, J Urol 33 254, 1935
- 15 Wessen, Miley B Discussion on paper of Heckel, etc, J Urol 33 298, 1935
- 16 Herrold, Russell D Idem, pp 301, 302
- 17 Boerner, Fred, and Solis Cohen, Myer A Study of Pathogen Selective Cultures in Relation to Vaccine Therapy, Am J Clin Path 3 125, 1933
- 18 Lowe, E Cronin Pathogen Selective Cultures as an Aid to the Diagnosis of Infective Foci, Brit M J 2 98 1928
- 19 Solis Cohen, Myer Accentuating Pathogenic Organisms in Culture by Utilizing the Inhibitory Influence of Whole Blood Brit J Exper Path 8 149, 1927
- 20 Idem The Pathogen Selective Vaccine Its Preparation and Administration, Med Times 58 206, 1930
- 21 Solis Cohen, Myer, and Heist George D A Method of Distinguishing From Among Various Microorganisms Present in a Patient, Those That Are and Those That Are Not Acted Upon by That Patient's Whole Coagulable Blood Penn M J 25 27, 1921
- 22 Solis Cohen Myer and Rubenstone A I The Technique of the Pathogen Selective Method of Culture J LAB & CLIN MED 11 881, 1926
- 23 Wright, Almuth E, in conjunction with Morgan, W Parry, Colebrook, L, and Dodgson, R W Observations on Prophylactic Inoculation Against Pneumococcus Infections, and on the Results Which Have Been Achieved by It, Lancet 1 1 and 87 1914
- 24 Heist, George D, Solis Cohen Solomon, and Solis Cohen, Myer The Bactericidal Action of Whole Blood With a New Technique for Its Determination, J Immunol 3 261, 1918

25. Bull, C. G., and Bartual, Louis: Pneumococcus Cultures in Whole Fresh Blood: I. The Retardative Effect of the Blood of Immune Animals and the Mechanism of the Phenomenon, *J. Exper. Med.* 31: 233, 1920.
26. Smiley, H. Everett: Bactericidal Action of Blood of Rabbits Immunized Against Pneumococci, *J. Infect. Dis.* 33: 88, 1923.
27. Robertson, Oswald H., and Sia, Richard H. P.: Studies on Pneumococcus Growth Inhibition. II. A Method for Demonstrating the Growth Inhibitory and Bactericidal Action of Normal Serum-Leucocyte Mixtures, *J. Exper. Med.* 39: 219, 1924.
28. Matsunami, Taitso, and Kolmer, John A.: The Relations of the Meningococcal Activity of the Blood to Resistance to Virulent Meningococci, *J. Immunol.* 3: 201, 1918.
29. Malone, R. H., Avari, C. R., and Naidu, B. P. B.: The Bactericidal Power of the Blood of Rats as a Measure of Their Immunity to Plague, *Indian J. M. Research* 13: 121, 1925.
30. Heist, George D., Solis-Cohen, Solomon, and Solis-Cohen, Myer: A study of the Virulence of Meningococci for Men and of Human Susceptibility to Meningococcal Infection, *J. Immunol.* 7: 1, 1922.
31. Beckley, A. G.: Administration of Vaccines, *Penn. M. J.* 32: 888, 1929.
32. Crowe, H. Warren: The Specific Vaccine Treatment of Chronic Arthritis and Rheumatism, *J. LAB. & CLIN. MED.* 15: 1072, 1930.
33. Graham, R. V.: Some Principles Underlying the Diagnosis and Treatment of Focal Infections With Particular Relation to Arthritis, *Med. J. Australia* 16 (2): 665, 1929.
34. Lowe, E. Cronin: Foci and Nature of Infection in 100 Cases of Rheumatic Conditions, *Brit. M. J.* 2: 43, 1929.
35. Shearman, C. H.: The Application of Pathogen-Selective Methods of Culture in the Investigation of Foci of Infection in Arthritis, *Med. J. Australia* 16 (2): 662, 1929.
36. Thomson, David, and Hill, N. Gray: Recent Researches on the Streptococcal Etiology of Rheumatic Fever, *J. LAB. & CLIN. MED.* 15: 1124, 1930.
37. Walls, R. M.: The Pathogen-Selective Method of Culturing, *Dental Cosmos* 67: 806, 1925.
38. Ramsay, Jeffrey, and Pearce, C. M.: Tonsil Puncture: A New Method of Investigation, *Brit. M. J.* 1: 543, 1929.
39. Barlow, D. L.: Vaccine Therapy, *Med. J. Australia* 17(1): 671, 1930.
40. Kolmer, John A.: The Present Status of Vaccine Therapy. Principles Involved; Analysis of Cases of Success and Failure and Results of Vaccine Therapy in Prophylaxis and Treatment of Disease, *Atlantic M. J.* 31: 208, 1927.
41. Solis-Cohen, Myer: Some Interesting Pediatric Cases With a New Method of Bacteriological Study and Treatment, *New York Med. J.* 112: 967, 1920.
42. Boerner, Fred, and Mudd, Stuart: Determination of the Phagocytic Power of Whole Blood or Plasma-Leukocyte Mixtures for Clinical or Experimental Purposes, *Am. J. M. Sc.* 189: 22, 1935.
43. Flemming, Alexander: The Bactericidal Power of Human Blood and Some Methods of Altering It, *J. Laryng. & Otol.* 43: 385, 1928.
44. Hare, Ronald: The Haemolytic Streptococci From the Vagina of Febrile and Afebrile Parturient Women, *J. Path. & Bacteriol.* 38: 129, 1934.
45. Mackie, T. J., Finkelstein, M. H., and Van Rooyen, C. E.: The Comparative Bactericidal Action of Normal Serum, "Whole" Blood and Serum-Leucocyte Mixtures; With Further Observations on the Bactericidal Mechanism of Normal Serum, *J. Hyg.* 32: 494, 1932.
46. Cabot, Hugh, and Crabtree, E. Granville: The Etiology and Pathology of Non-Tuberculous Renal Infections, *Surg. Gynec. Obst.* 23: 495, 1916.
47. Wood, V. V.: The Paranasal Sinuses as Sources of Infection, *Ann. Otol., Rhin. & Laryng.* 37: 592, 1928.
48. Horder, Thomas: *Bacillus Coli* Infections. In a Textbook of the Practice of Medicine. Ed. by Frederick W. Price, ed. 2, 1926, London, Milford, p. 79.
49. Young, Hugh H., and Davis, David M.: *Practice of Urology* 1: Philadelphia, 1926, W. B. Saunders Co., p. 96.
50. Chown, Bruce: Pyelitis in Infancy: A Pathological Study, *Arch. Dis. Child.* 2: 97, 1927.

THE WEIGHT LOSS OF TUBES OF CERTAIN PATHOGENIC FUNGI GROWING ON A SPECIFIC MEDIUM*

JOHN W. WILLIAMS, M.D., AND LEO GREEN, B.S., CAMBRIDGE, MASS

WE HAVE been unable to find reference in the literature to weight loss of tubes of medium with the lapse of time under stated climatologic conditions. This paper deals with a study of weight loss, using planted and unplanted tubes. We have used our routine medium for pathogenic fungi. It is labeled W medium (1 per cent dextrose, 4 per cent peptone, 1½ per cent agar adjusted to pH 5.8) and is superior to Sabouraud's proof medium in the gross study of these organisms since growths as a rule are more delimited, more constant in type and show less pleomorphism.

Planted and unplanted tubes were placed with slants upright in diffused light and allowed to remain at room temperature. The initial weight was taken and they were weighed after the following intervals: 2, 5, 7, 9, 14, 19, and 35 days (July 11, 1934 to Aug 15, 1934). All possible care was taken in handling the test tubes, cotton plugs and plants. Small bits of growth of as near as possible the same size were planted. The tubes were of the same size (2 x 15 cm) and the medium in each exactly 10 cc (9.513 gm). The medium was sterilized by autoclaving at 15 pounds' pressure for twenty minutes and slanted at 25 degrees. The data tabulated in Fig 2 were the average of 3 tubes. The greatest variation from the mean of the 3 tubes was 10 per cent and the least 0.4 per cent.

The following pathogenic fungi and 2 saprophytes, *Lachthermia* sp and *Scopulariopsis brevicaulis*, were planted. *Achorion schoenleinii*, *Acladium castellanii*, *Candida candida*, *Endodermophyton tropicale*, *Endomyces capsulatus*, *Endomyces dermatitidis*, *Epidermophyton cruris*, *Epidermophyton inguinale*, *Glenospora gammeli*, *Geotrichum bachmanni*, *Indiella americana*, *Microsporon audouinii*, *Microsporon felineum*, *Microsporon gypsum*, *Monosporum apiospermum*, *Monilia albicans*, *Oöspora humi*, *Sporotrichum schenckii*, *Trichophyton crateriforme*, *Trichophyton granulosum*, *Trichophyton gypsum asteroides*, *Trichophyton gypsum laticolor*, *Trichophyton japonicum*, *Trichophyton interdigitale*, *Trichophyton niveum*, *Trichophyton sulfureum*, *Willia anomala*.

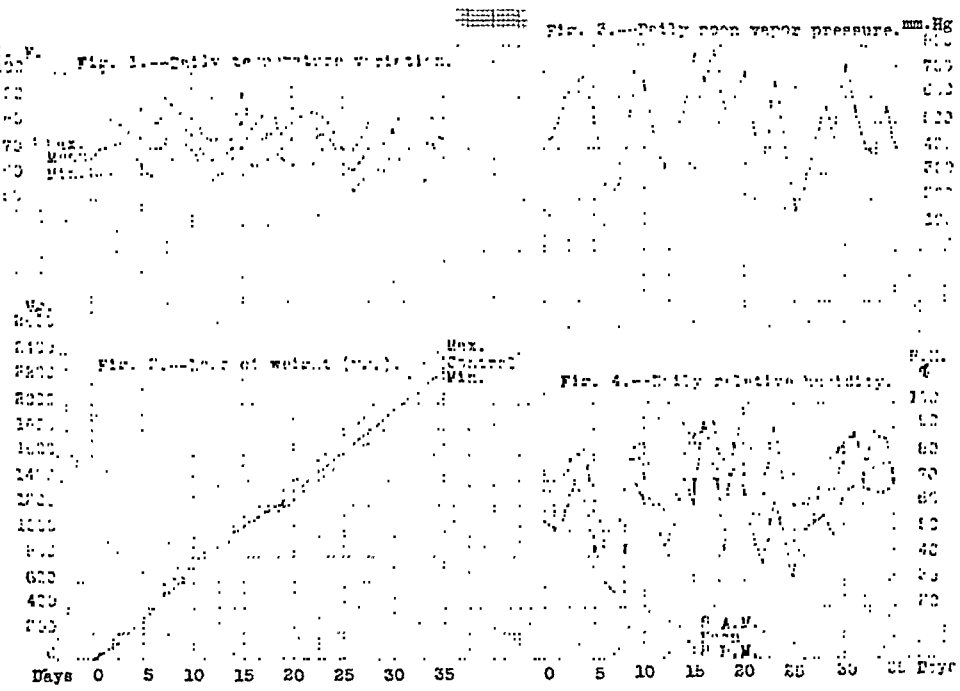
The interrupted lines in Fig 2 illustrate the maximum and minimum decrease in weight. The continuous line represents the controls of unplanted medium. The mean loss of weight per tube is approximately 0.065 gm, or 7 mg per gram of medium per day. Our original medium contained approximately 0.15 gm agar, 0.4 gm peptone, 0.1 gm dextrose in 10 cc. The average weight

*From the Department of Biology and Public Health and the Homburg Memorial Infirmity, Massachusetts Institute of Technology.
Received for publication July 8 1935

of 10 c.c. medium was 9.513 gm., of which 0.65 gm. were the above and the balance, or 8.86 gm., water. The water loss for thirty-five days was about 2.3 gm. (25 per cent) resulting in a concentration of constituents of from 4 to 5 per cent.

Fig. 1 illustrates the temperature change from day to day during the period of study. Fig. 3 illustrates the daily noon vapor pressure and Fig. 4 the daily relative humidity. These factors undoubtedly are important since a study¹ for another purpose revealed a weight loss on an average of 8.6 mg. per gm. per day in place of 7 mg. per gm. per day as found in this study.

The foregoing data reveal that these organisms play little part in the retention or dissipation of moisture. Apparently, a certain part of the medium is



converted into organism. The weight loss of the planted and unplanted tubes is strikingly similar. The amount of water loss for this medium should correspond fairly consistently with water loss in other media. These tabulations should make the laboratory worker realize that he is actually planting his organisms on different media when he uses fresh and several-week-old media, due to the variation of percentage of ingredients.

REFERENCE

1. Williams, J. W.: Proc. Soc. Exper. Biol. & Med. 32: 918, 1935. Contribution No. 71 from the Department of Biology and Public Health, Massachusetts Institute of Technology, Cambridge, Mass.

HEMOGLOBIN VALUES IN NORMAL ADULTS OVER A PERIOD OF TIME*

WINIFRED INGERSOLL, MADISON, WIS

THE purpose of the present study was to determine the range and variations in hemoglobin values in normal adults over a period of time. This information is important because the significance of a single value depends upon whether the factor is a constant or variable one in the apparently normal individual. At the present time few data are available and the results are not consistent. Rud¹ states that hemoglobin values in human subjects are even less variable over periods of time than those of red blood cells. A study of the data of Reich and Green² shows considerable variation in each of six female subjects.

METHOD

The subjects of the present study were thirty young adult medical students. Determinations were made with a Bausch and Lomb hemoglobinometer after the method of Newcomer. A constant source of light was used. Samples of blood were taken from the ear lobe in a few cases from the fingers. A lens was used to read the meniscus. As far as possible, determinations were made weekly or oftener during the months of October to January, and no subject had less than ten. The ten subjects who showed rather steady declines were rechecked in the spring and six of them again after one year. A record was kept in each case of age, height, weight, personal habits, and the occurrence of menstruation and illnesses.

DATA

A study of the individual graphs which cannot be included because of their bulk, reveals the following facts. First the difference between the maximum and minimum hemoglobin values for the period or the range varies for the different subjects between 0.4 and 2.7 gm per 100 cc of blood. Fourteen subjects have a range of 1.0 gm or less and sixteen a range of over 1.0 gm. Females show greater differences than males, the average respective ranges being 1.54 gm and 1.10 gm. Second, the variations from the average for the subject were determined for each hemoglobin reading and the distribution of variations shown by means of frequency polygons (see Fig 1). It will be noted that for the group as a whole small variations of 0.1 and 0.2 gm are most likely to occur, while for the males the most frequent variations are 0.1, and for females 0.2 and 0.5 gm. Also, as to the general trend over the period 41 per cent of the group show irregular trends though in many cases the final values are lower than the

*From the Physiological Laboratory, University of Wisconsin.
Received for publication July 19, 1935.

initial, in a few cases slightly higher, and in the rest about the same as at the beginning. Thirty-three per cent show a tendency to constant values and 26 per cent a definite downward trend. Determinations made in the spring upon the latter show values very similar on the whole to those of the winter months. In no case was the high fall value completely regained. In October, 1934, one year after the original determinations, one of the six available subjects shows a lower

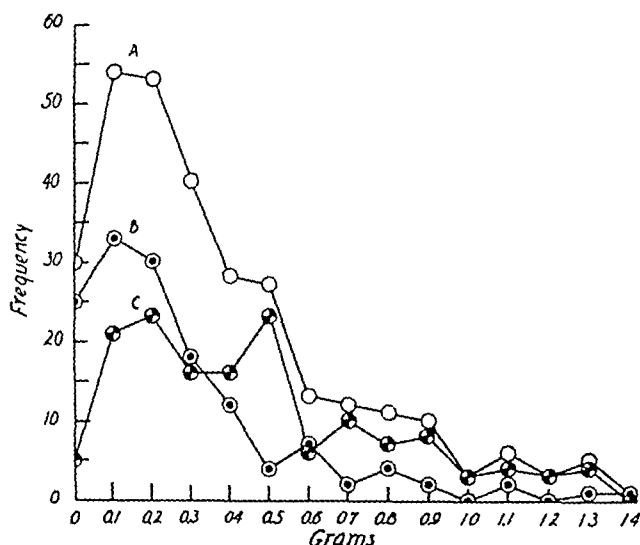


Fig. 1.—Frequency polygons showing distribution of variations from average hemoglobin value for the subject in grams per 100 c.c. blood. A, total; B, males; C, females.

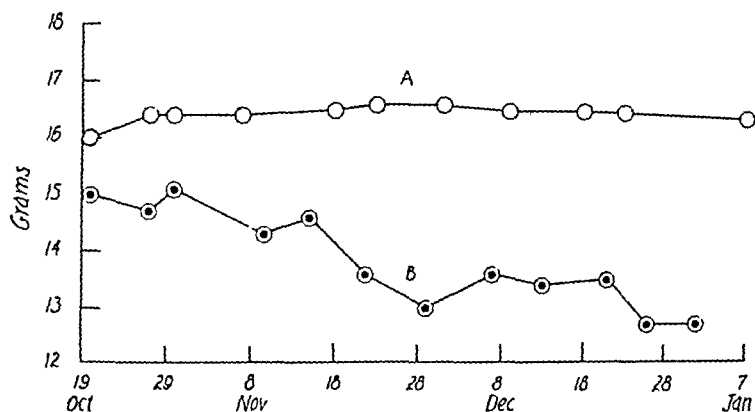


Fig. 2.—Hemoglobin values in grams per 100 c.c. blood for two subjects, A, male, No. 8; B, female, No. 10, showing the differences which may occur in range, weekly variations, and general trend of values.

value than at any previous time, one is still at the spring level, and four are as high as they were the preceding October. Finally, there is no apparent correlation between build or habitual number of hours of sleep and average hemoglobin values. Most of the illnesses reported were colds. The more severe ones are accompanied by a change which occurs irregularly before or after the illness. No consistent relation is evident between hemoglobin variations and menstruation.

DISCUSSION

According to the data individuals differ as to their hemoglobin picture. While some tend to maintain a rather constant level over a period of time, others vary considerably as to range, weekly values, and general trend. Graphs 8 and 10 (Fig 2) well illustrate this point. While No 10 happens to be one of the more variable of the female subjects, two of the males show as great a range. Any reading from No 8 would give an accurate idea of the condition as to hemoglobin, while a single reading from No 10 would be quite misleading. These facts show the absurdity of relying upon a single reading in clinical studies. Information which the subjects were able to give was inadequate in most cases to explain their dissimilarities. The marked sex differences suggest menstruation as a possible factor. No consistent relation has as yet been reported between its various phases and hemoglobin changes, but before it is ruled out a more extensive study should be made with frequent determinations before, during, and after the period. It is perhaps noteworthy that the only female included in the "constant group" is a case of prolonged amenorrhea. Other factors which may have played a part are the following. Females omitted meals somewhat more frequently and missed whole or part nights of sleep almost twice as often, and while males reported more illnesses, females had all the more severe ones requiring absence from classes.

A downward trend between the months of October and January is present in about 50 per cent of the group and marked in 26 per cent. Seasonal variation has been reported by some workers³ and is a factor to be considered here since four of the six subjects who were observed for a year showed a return to their original values in the corresponding months of the second year.

CONCLUSIONS

- 1 Individuals who are apparently normal show marked differences as to the range, weekly variations, and general trend of their hemoglobin values.
- 2 In the six subjects observed for one year, seasonal variation is suggested.
- 3 Females vary more than males.
- 4 A single hemoglobin determination may be misleading. For that reason several determinations should be made as a basis of diagnosis or treatment.

REFERENCES

- 1 Rud, E. J. Le nombre des globules rouges chez les sujets normaux et leurs variations dans les diverses conditions physiologiques. *Acta Med Scandinavica* 57: 142, 1922-23.
- 2 Reich, C., and Green, D. Red Cell Regeneration During the Menstrual Cycle, *Arch. Int. Med.* 49: 534, 1932.
- 3 Lippincott, L. S. Hemoglobin and Erythrocytes in the South, *J. Lab. & Clin. Med.* 12: 679, 1927.

STUDIES ON *T. VAGINALIS* IN VITRO*

H. A. SHELANSKI, A.B., PHILADELPHIA, PA.

THIS study is a part of a larger investigation of methods of culturing and controlling various species of *Trichomonas*, which includes *T. vaginalis*, *T. buccalis*, *T. hominis*, *T. batrachorum*, and four strains of *Trichomonas* from the snake. The scope of this report is limited to methods of cultivation and of control in vitro of *Trichomonas vaginalis*. The purpose in reporting this part of the investigation is to make available to those interested a technic which has been found satisfactory for detecting, cultivating, and studying the properties of this parasite. The series of studies on *T. vaginalis* was carried out on a culture originally derived from a case of chronic vaginitis of the following history:

Mrs. L. A., white, para iii, six months pregnant, vaginal mucosa was inflamed and hemorrhagic. Vaginal secretion was profuse, yellow, thin, and bubbly. Clinically the case was a typical instance of trichomonas vaginitis. Wet smear showed *Trichomonas* in large numbers.

The method of obtaining the primary culture was as follows:

The vagina was distended by means of a speculum and some material was gathered from the region of the posterior fornix with a sterile swab, which was then placed in a test tube containing 5 c.c. of Ringer's No. 2 solution.

This specimen was inoculated into a culture medium consisting of 0.1 per cent Loeffler's blood serum in Ringer's No. 2 solution, which contains:¹

NaCl	6.0 gm.
KCl	0.1 gm.
CaCl ₂	0.1 gm.
NaHCO ₃	0.1 gm.
In 1,000 c.c. of distilled H ₂ O.	

To confirm the presence of *T. vaginalis* in a specimen a small drop, which should be perfectly liquid and fresh, is placed on a micro slide and examined under the low power objective, and confirmed by examination under the high power dry lens. As soon as possible after taking the fresh material from the vagina and not longer than two hours, 1 c.c. of the fresh vaginal swab (Ringer) specimen is added to 10 c.c. of culture medium. This is incubated at 37.5° C. After forty-eight hours this culture (10 or 11 c.c.) is added to 500 c.c. of culture medium and again incubated at 37.5° C. The organisms appear in fairly large numbers after two days of incubation, and continue to exist in this state for about one week.

In the study being reported here this procedure yielded a viable culture which contained approximately 750 individual organisms per cubic centimeter. The organisms showed the characteristic form and motion. Upon examination

*From the Zoological Laboratories, University of Pennsylvania.
Received for publication, July 19, 1935.

under the high power dry lens the organisms proved to be very healthy and active with hardly any distortion. At lethal concentrations of silver picrate, silver nitrate, strong and mild silver protein, organisms ceased their motion and became distorted, then the undulating membrane also ceased its motion and the organism became quiescent. Shortly after this the body membrane ruptured and its contents disappeared in the medium. In the case of lethal concentrations of picric acid and sodium picrate the organism assumed a perfectly spherical appearance and after some time, the membranes burst.

The compounds studied were picric acid, silver nitrate, silver picrate,* silver protein strong, silver protein mild, and sodium picrate. Each of these substances was used in four dilutions viz, 1:100, 1:500, 1:1,000, and 1:5,000, in distilled water. Addition of these dilutions to an equal volume of culture produced a final effective concentration one half that of the stock dilution. To 5 c.c. of the culture containing living flagellates 5 c.c. of the solution to be tested was added. Five tests were made for each dilution of all the substances. In order to evaluate the "buffering" effect of the Loeffler blood serum a parallel series was run in which the Loeffler Ringer medium was removed by centrifugation and the organisms taken up in Ringer's No. 2, after which the compounds under investigation were added in the usual way. This control series showed that there was a distinct "buffering" or protective action of the culture medium. Freeing the organism from culture medium and suspension in Ringer's No. 2 solution rendered them more easily susceptible to the action of the substance.

EFFECT IN CULTURE MEDIUM (NOT CENTRIFUGED)

SUBSTANCE TO BE TESTED	CONCENTRATION OF TEST SOLUTION ADDED TO CULTURE	TIME TO KILL	
		AVERAGE OF FIVE TRIALS	RANGE
Picric acid	1:100	10	10
	1:500	30-40	30-10 to 32-40"
	1:1,000	51-30	50-10 to 52-30'
	1:5,000	1 hr 16-30	1 hr 15-30 to 1 hr 17-30
Silver nitrate	1:100	10	10
	1:500	4-50	4-30 to 5-20"
	1:1,000	6-50"	6-30 to 7-0
	1:5,000	9-2	8-10 to 9-40'
Silver picrate	1:100	10	10
	1:500	51	55 to 1-10
	1:1,000	3-32	3-10 to 3-40
	1:5,000	5-58	5-30" to 6-30
Protargol	1:100	10	10
	1:500	4-30	3-50 to 5-10'
	1:1,000	21-52	20-30 to 23-40
	1:5,000	24 hr	24 hr
Argyrol	1:100	8-30	8-10" to 9-20"
	1:500	26-30'	25-30" to 27-40
	1:1,000	46-20"	45-30 to 47-50"
	1:5,000	24 hr	24 hr
Sodium picrate	1:100	10"	10
	1:500	4-30	4'-10 to 4-50"
	1:1,000	21-8	20-10" to 22-30"
	1:5,000	24 hr	24 hr

*Silver picrate $\text{AgC}_6\text{H}_3\text{O}_7 \cdot \text{Na}_2\text{H}_2\text{O}$ (353-94) containing 30.48 per cent silver was supplied through the courtesy of John W. Jeth & Bro. Inc. Philadelphia.

being tested. The time required to kill the organisms was recorded as that time when they were found, upon examination of three successive drops, to be all dead. Only when all three examinations showed no living animals was the time recorded accepted. As a control for these experiments 5 c.c. of distilled water were added to 5 c.c. of culture, and to 5 c.c. of the centrifuged material suspended in Ringer's No. 2 solution. This control was used for all of the species of *Trichomonas* examined. No change was evident in the activity of the organisms in the culture medium diluted with distilled water for five days. In the centrifuged material no change was evident after three days. Therefore, the effect on the organisms of the distilled water in which the chemicals are dissolved may be ignored.

TRICHOMONAS VAGINALIS

1. Five cubic centimeters of test solution were added to 5 c.c. of culture medium: thus the concentration of the test solution was cut in half.
2. The test solutions are all aqueous solutions.
3. The time to kill the organisms is taken as the time between the addition of the test solution and the time when all the organisms were found to be dead.
4. Ten seconds is the time between the addition of the test solution and the examination.

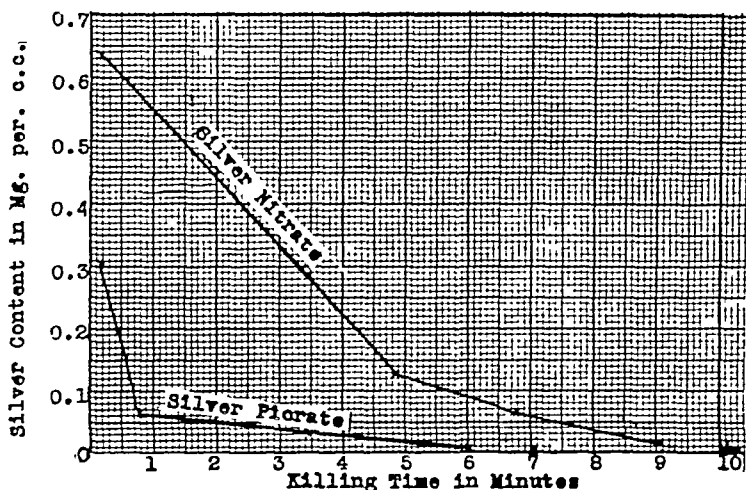


Fig. 1.—Graph of killing time of silver nitrate and silver picrate on *Trichomonas vaginalis* in vitro.

The results of comparison of the effect in vitro of silver picrate with silver nitrate, recalculated in terms of the lethal concentration of metallic silver, are shown in Fig. 1. If the separate rôle of the cation is not taken into account it will be noted that silver picrate is effective at lower silver concentration. Strong silver protein shows a killing power very similar to that of silver picrate. The mild silver protein is relatively ineffective, showing its first effect at a concentration of 0.25 mg. silver per c.c. in eight and one-half minutes.

SUMMARY AND CONCLUSIONS

Details are shown of a satisfactory method for obtaining and cultivating viable strains of *Trichomonas vaginalis* in amounts suitable for determining the effect of various substances upon the organism.

An example of such a study is shown, consisting of the determination of the lethal concentration of various silver compounds, including silver picrate, silver nitrate, and the mild and strong silver proteins

Solutions of silver picrate destroy *T. vaginalis* at low concentrations of silver

The author wishes to express his appreciation to Dr David H Wenrich for guidance during the course of this investigation

REFERENCE

- 1 Drbohlav, J J Une nouvelle preuve de la possibilité de cultiver *Endamoeba dysenteriae* type histolytica Annales de Parasitologie Hum et Comp 3 349, 357, 1925

A CASE OF MENINGITIS IN A NEWBORN INFANT DUE TO A SLOW LACTOSE FERMENTING ORGANISM BELONGING TO THE COLON BACILLUS GROUP*

M E MULHERN, B S AND WALTER BALF SEELAE, M D, SEATTLE WASH

THE patient, a male child, the second sibling of healthy young parents, was first seen on May 28 1934 at the age of five weeks The pregnancy had been uneventful and the baby was born at term, a normal delivery Breast feeding was tried for only two weeks then a dilute milk water and dextri maltose formula was given Orange juice and cod liver oil had been started at three weeks and were being given daily The complaint at this time was that the infant seemed dissatisfied with the feedings and was somewhat irritable Physical examination revealed a thin, somewhat poorly nourished male infant with a normal temperature The length was twenty one inches, the weight seven pounds, fourteen ounces The posterior fontanel was closed, the anterior fontanel one and one half centimeters in diameter, soft, level, no pulsations noted The eyes, ears, mouth, and pharynx were normal The heart was not enlarged to percussion, its rate and rhythm were normal and no murmurs were heard The lungs were clear and resonant throughout The abdomen was soft, level, with no palpable masses and the liver, spleen, and kidneys were not felt The umbilicus was healed and presented no hernia The genitalia were normal A whole milk, water and dextri maltose formula was prescribed with five feedings at four hourly intervals the cod liver oil and orange juice to be continued as before

One week later there had been no gain in weight and the infant had a weak, whimpering cry, high pitched and constant Kernig's sign was negative There was no neck rigidity and the spine flexed easily, though he lay for the most part with his head retracted and back arched The bowels were loose and frequent and the mother had noted some mucus in the stool A small cerebral hemorrhage was considered as a possible diagnosis at this time, and it was felt best to continue conservative treatment An evaporated milk, water, and karo formula was

substituted for the whole milk and dextri-maltose mixture and the mother was cautioned to report immediately any change in the child's condition. Two days later, after a short interval of improvement, the infant's condition was definitely worse. The rectal temperature was 100° F., the heart rate 180, and a short systolic murmur was heard at the cardiac apex. The infant lay in oposthotonos, the fontanel was bulging, the patellar tendon reflexes were exaggerated and Kernig's sign was positive bilaterally. A tentative differential diagnosis was made of cerebral hemorrhage or meningitis and spinal puncture was performed. Eight cubic centimeters of yellowish, cloudy, blood-tinged fluid were withdrawn under no apparent increase in pressure. Five cubic centimeters of antimeningococcic serum were given intraspinally.

A clot formed in the test tube within a few minutes. The smear showed no organisms; the cell count was 12,000 with 94 per cent polymorphonuclear cells, sugar 9.4 mg., Wassermann negative. The patient was admitted to the Children's Orthopedic Hospital. During the succeeding twenty-four hours there were repeated slight generalized convulsions and the fontanel became more and more tense. Spinal puncture was attempted the following day but no fluid was obtained. Cisternal puncture yielded 15 c.c. of cloudy, yellowish fluid under moderately increased pressure. The next day the spinal and nuchal rigidity were pronounced, the fontanel was bulging, the patellar reflexes were absent, the abdomen was distended and tympanitic, there was drooling at the mouth, and the pupils were contracted. The parietal sutures were beginning to separate. There were two slight, generalized convulsions during the night. The baby continued taking his formula and water with no vomiting throughout the course of the illness so that at no time was there any need for the parenteral injection of fluids.

From this point on, the course was steadily downhill, the convulsions coming more frequently and becoming more severe each day. On the third day the laboratory to which spinal and cisternal fluid specimens were sent reported a pure culture of an organism which, as far as could be classified, fell within the paratyphoid group. Toward the last the stools became liquid and quite frequent. Cultures from one specimen were negative for the paratyphoid group.

Further questioning subsequent to the time the baby was first seen elicited the information that the mother had suffered from a small breast abscess when the baby was three weeks old. This was apparently a minor affair, and when it is remembered that the infant had been weaned at two weeks, it becomes plain that there was no etiologic connection here with the child's subsequent infection. Attempts were made to have urine and stool cultures made from the mother, father, and housemaid, but we were unsuccessful due to a lack of cooperation and failure on their part to realize the scientific importance of such tests.

The temperature of the patient ranged between 99° F. and 104° F. and the general downhill progression was interrupted only by days of apparent but short-lived improvement. Five days after admission to the hospital the cisternal fluid became so thick it would not run through a needle of large bore, resembling pure pus in its appearance. The punctures were done up until this time at intervals of from twelve to twenty-four hours depending on the evidences of pres-

sure from day to day. The child became comatose and during the last three days of life could not be aroused from this stupor though strangely enough he retained the ability to suck from a bottle up to the last. Death occurred during the night, three weeks after he was first seen. Permission for postmortem examination was not granted.

BACTERIOLOGY

Morphology of culture—*Macroscopic* Grew readily on dextrose blood agar, chocolate agar, hormone blood agar, and endo. On endo the colonies were colorless. On Russell's triple sugar medium it gave an acid and gas butt and alkaline slant. (Reaction of *Salmonella* group—paratyphoid.)

Microscopic The organism gram negative, short rod, showing coccobacillary forms with occasional longer filamentous forms actively motile.

Serologic Reactions Macroscopic agglutination tests made with the organism and specific agglutinating serums for *B. typhosus*, para A, para B, *B. Acetivrycke*, *B. Supestifer*, and *B. enteritidis* gave no agglutination, dilutions used from 1:20 through 1:320. The patient's serum tested with culture of *B. typhosus*, para A, para B, *B. Acetivrycke*, *B. Supestifer*, and *B. enteritidis* gave no agglutination.

Agglutination of 1:200 was obtained with the patient's serum and the organism from the spinal fluid.

Pathogenicity Guinea pig and mouse injected intraperitoneally died in forty-eight hours. Guinea pig and mouse injected subcutaneously appeared normal at the end of five days. Guinea pig and mouse fed organism appeared normal at the end of five days.

Biochemical Reaction Galactose, lactose, xylose, maltose, dextrose, mannite, rhamnose, trehalose, arabinose were fermented with production of acid after twenty-four hours' incubation. Dextrin, inulin, lactose dulcitol, sucrose, raffinose, inositol were not affected at the end of ninety-six hours. There was no production of gas in lactose broth.

The culture was then sent to Dr. G. W. McCoy, Director of the National Institute of Health, Washington, D. C. He reported back that the organism belonged to the slow lactose fermenting group of *B. coli*. In his letter reference was made to Wilson's statement "That there is some relationship between the power to ferment lactose and virulence is suggested by the fact that the pathogenic paratyphoid group are nonlactose fermenters and also by Dudgeon's account (1924) of forty-nine cases of very severe acute infections of the genitourinary tract in which all the strains of *B. coli* showed delayed fermentation of lactose."

After receiving this report we again repeated the sugar reactions, a period of six weeks having elapsed since the organism was isolated. Lactose was fermented at the end of fourteen days.

REVIEW OF THE LITERATURE

In view of the fact that the organism culturally resembled the paratyphoid group, the literature was examined with the idea that some of these cases in which the organism isolated did not agglutinate with known serum might possibly belong to the slow lactose fermenting group of *B. coli*.

The first case of paratyphoid meningitis to be recorded in the English literature is reported by Brahdya³ in 1925. This is a case of purulent meningitis due to *B. paratyphosus B* in a child of thirteen months. The infection started with a slight gastrointestinal disturbance which was complicated by a bronchopneumonia and a meningitis one week after onset. The cerebrospinal fluid contained a single strain of bacilli which formed acid and gas in glucose broth and did not ferment lactose. Agglutination reactions with paratyphosus B serum were positive to dilutions of 1:2,700. Stool cultures were negative. The author reports mention of a case of paratyphoid meningitis in a child by Ghon in 1907 at the Kongress für Hygiene, and two cases reported by Arzt and Bose in 1908. One of these latter occurred in an infant five months old, due to paratyphoid B; the other in an infant seven weeks old in which the infecting organism was found to be morphologically and biologically similar to the paratyphoid bacillus but which did not give the agglutination reactions characteristic of that organism. It is this case which seems most similar to our case. This author also mentions other cases by Hundshagen and Voigt who each reported a single case due to paratyphosus B. Blechmann is said to have reported two cases in infants with recovery in both, in which agglutination tests with the bacilli in the spinal fluid were positive only with paratyphosus B serum. In this same article mention is also made of a case reported by Walterhofer in 1917 in a soldier twenty years old. Paratyphoid B bacilli were cultured from the spinal fluid on three successive days, after which the fluid was sterile. The stools remained negative during the meningeal infection.

Gatewood⁴ records the case of a thirteen-year-old boy who received a head injury when at a year and a half of age he fell from a porch. A skull fracture at this time was followed by jacksonian epilepsy for the relief of which an osteoplastic flap was turned down and several spurs of bone removed from the dural side of the flap. This was followed by the development of a meningitis. The cerebrospinal fluid contained *Bacillus fecalis alkaligenes*. No further treatment was instituted and the patient made an uneventful recovery. The author states that according to Stitt⁵ *B. fecalis alkaligenes* is a frequent inhabitant of the intestinal tract, and it has been isolated from the blood of a few cases resembling typhoid fever. Topley and Wilson⁶ refer to work done in 1896 by Petruschky who described the *Bacillus alkaligenes* as "an inhabitant of the human intestine with pathogenic potentialities."

Jundell⁷ reports a case of a two-month-old breast-fed male infant who was admitted to the hospital with a history of vomiting, fever and loose stools of sudden onset beginning the day before admission. The mother had been quite well and had had normal stools. Spinal puncture gave a dry tap and ventricular puncture was done, yielding 40 c.c. of thick, cloudy, reddish brown, fetid fluid, containing predominantly polymorphonuclear cells. This contained paratyphoid B bacilli, which organism was also cultured from the urine. The stools were negative and agglutination tests on the blood were negative for typhoid, paratyphoid A and B, and the Gartner and Bang bacillus. The child died after an illness of six weeks. The mother's blood gave a faintly positive agglutination test for paratyphosus B: her stools were positive for the same organism and the breast milk gave a faintly positive agglutination reaction.

Ratcliffe⁸ adds a case of a six-week-old female infant who was admitted to the Children's Hospital, Sheffield, on Feb. 1, 1935, with a history of "convulsions at intervals for twelve days." The onset was sudden, there was no history of birth injury and she was a breast-fed baby. Examination revealed a hydrocephalic head with the sutures all widely opened and the fontanel bulging. Kernig's sign was positive, the pupils reacted sluggishly to light, the abdominal reflexes were absent, the legs very spastic, and the knee jerks accentuated. Lumbar puncture was done and 2 c.c. of clear fluid withdrawn under no increase of pressure. Culture of this gave a pure growth of *B. coli*. The child's condition grew progressively worse and death occurred twelve days after admission to the hospital. Postmortem examination showed a large brain with distended ventricles "filled with semimucoid greenish pus, the cortex being reduced to paper thickness in places." This material was identified by direct smear as morphologically "Gram-negative organisms resembling *B. coli*."

In commenting on this case the author notes that *B. coli* meningitis "seems to be seen more often in children, especially infants, than in adults. Convulsions are usually a conspicuous feature but the signs are frequently equivocal and do not always point so clearly to a

meningitis. It is usually considered to be a terminal event in a *B. coli* septicemia and some focus of infection is usually found elsewhere in the body." Reference is made to a review of the literature in 1926 by Neal in which 42 cases were reported.

Neal states that in an analysis of "more than 1,500 cases of meningitis seen by the Meningitis Division of the Research Laboratory (New York City Department of Health) up to 1924, only five cases were found due to the *B. coli* in pure culture." She comments that children under six months of age suffering from meningitis are particularly prone to develop a subacute arachnoiditis. In a series of fifty cases of meningitis in the first three months of life, twenty-four were due to the meningococcus and only three to the *B. coli*.

SUMMARY

1. An organism isolated from a case of meningitis in a newborn infant resembled macroscopically and microscopically, the paratyphoid group.

2. The sugar reactions at the end of ninety-six hours' incubation were those of the paratyphoid group but no agglutination was obtained with specific agglutinating serums of this group.

3. After fourteen days' incubation of the sugars lactose was fermented, which classifies the organism in the slow lactose fermenting group.

4. A review of the literature reveals no other case in which this organism was the infecting agent as proved bacteriologically by the slow fermenting of lactose though several of the cases recorded have many points in common with our case and may well have been due to this same organism.

REFERENCES

1. Wilson, W. J. (Belfast) "Pathogenic Slow Lactose Fermenting *B. coli*." A System of Bacteriology (Medical Research Council) 4: 267, 1929.
2. Dudgeon, Leonard S. (London) quoted by Wilson, W. J. Acute Infection of the Urinary Tract Due to a Special Group of Hemolytic Bacilli, *J. Hygiene* 22: 348, 1923-24.
3. Brahm, M. Bernard. A Case of Meningitis Caused by the Bacillus Paratyphosus B, *Arch. Pediat.* 42: 550, 1925.
4. Gatewood. Bacillus Feerle Alkaligenes Meningitis, *Am. J. Surg.* 12: 435, 1931.
5. Stitt, E. R. Practical Bacteriology, Blood Work and Parasitology, ed. 5, 1927, p. 179. Quoted by Gatewood.
6. Topley, W. W. C., and Wilson, G. S. The Principles of Bacteriology and Immunity, 1. New York, 1929. W. Word and Co. p. 419.
7. Jundell, I. A Case of Paratyphoid Meningitis, *Acta Paediatr.* 14: 229, 1932.
8. Batchiffe, T. A. A Case of *B. Coli* Meningitis, *Lancet* 1: 1274, 1933.
9. Neal, Josephine B. Meningitis Caused by Bacilli of the Colon Group, *Am. J. M. Sc.* 172: 740, 1926.

1705 FOLLETH AVENUE
COBB BUILDING

PHOSPHORUS METABOLISM^{*}

VI. CHANGES AND RELATIONSHIPS IN BLOOD PHOSPHORUS OF RATS SUBJECTED TO BLOOD REGENERATION BY REPEATED BLEEDINGS

GUY E. YOUNGBURG, PH.G., PH.D., AND MAMIE V. YOUNGBURG, B.A.,
BUFFALO, N. Y.

THE object of the following work was to obtain new and more extensive data on the sources, levels and interrelationships of blood phosphorus compounds.

Studies on the type, distribution, and level of the phosphorus compounds in the blood of man and animals under normal, pathologic and special conditions are not few in number, yet the sources, relationships, functions, etc., are as yet little known. Such questions as the following are still unsettled: What is the immediate source for maintaining the inorganic phosphate of the blood? What are the functions of blood phospholipids and what is their relationship to the inorganic phosphate? How long can food phosphorus be inadequate without damage to the organism?

McGowan¹ adduced evidence to show that the probable mode of action of vitamin D in the cure and prevention of rickets is by setting free from the lipids of the body inorganic phosphate, the relative deficiency of which is regarded as the essential cause of rickets. However, he gave no direct evidence on the breakdown of phospholipids and no phospholipid determinations were made.

Muller and Heath² found that in anemia due to acute loss of blood, the lipoids remained at normal levels or were high. There was "no relation between the degree of anemia and the levels of the lipoids." Also "anemia is not directly related to the level of the cholesterol and the lecithin phosphorus of the blood." In anemia due to chronic hemorrhage lecithin phosphorus tended to be low when the anemia was severe.

In our laboratory³ we have found that human cancer bloods show certain changes in phosphorus distribution, but we could not be sure that they were not due to the accompanying anemia.

Heymann⁴ found that in children on a phosphorus-poor diet for only four days the plasma inorganic phosphorus diminished, accompanied by a rise of similar magnitude of the organic acid soluble phosphorus of the plasma.

In the research reported here it was thought that if an animal was deprived of its dietary phosphorus and at the same time if its body store or supply was depleted by successive bleedings, evidence would be obtained which would throw light on some of the questions indicated: especially would be shown the capacity to maintain the blood phosphorus compounds either by regeneration or by renewal by mobilization from other tissues.

^{*}From The Department of Biological Chemistry, University of Buffalo Medical School.
Received for publication, July 20, 1935.

We desired, if possible, to use the rat as the experimental animal, to draw sufficient blood at repeated intervals without especially endangering the animal, and to make the following determinations on the blood corpuscle volume, inorganic, organic acid soluble and lipid phosphorus on both corpuscles and plasma. Considerable time was spent in developing and perfecting technique, which included anesthetizing, drawing of the desired amount of blood, and the application of the microchemical methods. We were finally able to accomplish the drawing and analyses when rats weighing over 200 gm were used. It was difficult to get enough blood from smaller rats, and we did not want to pool bloods for this purpose. In the largest rats the tail veins are large enough to allow a very satisfactory blood flow. In rats of medium size (200 to 250 gm) sufficient flow is ordinarily obtained.

TECHNIQUE FOR REPEATED DRAWING OF RAT BLOOD

Our object was to remove as much blood from the animals as they would tolerate without endangering their lives or disturbing their ordinary food habits, etc., during an experimental period of from 15 to 20 days.

A rat weighing 250 gm has a total of about 15 cc of blood. If one removes one fourth to one third of this at each bleeding, it will amount to about 4 cc to 5 cc each time. By the method of tail bleeding described below such removal is possible with rats weighing over 200 gm. After a little experience there is no loss of rats and the injury to the tail does not affect the rat seriously, although the bleeding be repeated every third day for a period of at least eighteen days.

Procedure. The rat is put in a wire cage (of known weight) which will just comfortably contain it and the sliding door is closed. (A test tube basket provided with a sliding door can be used.) The total weight is obtained and then the cage with the rat is set aside until the anesthetizing gas is prepared. This is done by suspending a wad of cotton well moistened with chloroform and ether (1:1) by a wire hook on the upper inside wall of an ordinary cylindrical glass jar of about 8 inches in diameter and 6 inches high, large enough to contain the wire basket. The cover is placed on the jar and after several minutes the wire cage containing the rat is inserted and the jar cover replaced. By careful observation, just as soon as the rat is "under" it is quickly removed from the jar and placed on a holder,* the anesthetic is continued as necessary by the open method, using the cotton wad and as soon as the tail is insensible to sharp pressure, its end is cut off by means of a razor blade against a small block of wood. The tail is partly inserted into a 15 cc graduated centrifuge tube containing oxalate and a thin stirring rod. Gentle milking of the tail must nearly always be resorted to and the blood must occasionally be stirred in order to prevent coagulation. Usually 3 to 7 cc of blood are to be drawn, depending on the rat body weight.

*The holder may consist simply of a piece of board 12 inches by 7 inches by $\frac{1}{4}$ inches thick provided with sides and one end piece. The sides are strips 12 inches long and $\frac{1}{2}$ inches wide which are placed on top of the board and each is fastened at the top end by a single nail allowing adjustment in or out at the other end by means of holes and nail pegs. The adjustable width lower end of the board has a strip nailed to it the same width as the sides but with a notch 1 inch wide and equally deep for the protrusion of the tail. The board is supported by legs or otherwise at a slope of about 35° with the table top on which it is placed and about 6 inches from the table top. Although the rat is usually partly held in place by hand in this holder it is convenient and really necessary.

The rat is then immediately placed on a level surface and the end of the tail is dipped into concentrated tannic acid solution. No appreciable bleeding will result.

The depth of anesthesia must not be greater than is necessary; it is sufficient when the vibrissae no longer are motile and when the tail is insensible to the first razor blade pressure. If there is too deep, or too light, anesthesia, little blood can be obtained. Administration of the anesthetic can and should be regulated so that the animal will begin to regain consciousness almost as soon as the required amount of blood has been drawn.

ANALYTICAL METHODS USED

Two-tenths per cent potassium oxalate solution was used as anticoagulant. Corpusele volume was determined with Van Allen hematocrit tubes. The red corpuscles and plasma were separated by means of fine pipettes made by drawing out 1 cm. diameter glass tubing, fitted with rubber tubing for mouth suction. The white corpuscles were discarded.

Analyses of corpuscles and plasma for inorganic, organic acid-soluble and lipid phosphorus were made according to the colorimetric phosphomolybdic-acid-reduction method of Youngburg and Youngburg⁵ except as follows: as a rule only one-fifth quantities of the original methods were used. Since the quantities of corpuscles indicated were less than 0.5 c.c. they were weighed out in shallow tared glass vials (10 mm. outside diam. by 7.5 mm. high). These were then transferred into suitable graduated test tubes, no loss being incurred. The sp. gr. of 1.089 for corpuscles was used in the calculations. For quantities of plasma and reagents of 0.5 c.c. or less, we used 0.1, 0.2, 0.3, 0.4, and 0.5 c.c. micro-pipettes.* For larger quantities, tested serologic or Ostwald-Folin pipettes were used.

Since the final volumes of the solutions for color comparison were 2 c.c., we used a number of 100 by 13 mm. Pyrex test tubes which were graduated at 2 c.c. for this purpose. Tubes of smaller diameter are not satisfactory.

For the standard color solutions we used the original volumes, i.e., 0.02 mg. P in a total volume of 10 c.c.

On account of using volumes one-fifth of those of the original methods, the accuracy cannot be expected to be as good as was reported for the larger volumes. Practice analyses and many duplicate determinations during this research indicate a maximum of 4 per cent error.

EXPERIMENTAL

Animals Used.—The experimental animals were white rats weighing at least 200 gm., of unknown history, obtained at a local animal store. They were kept on our laboratory diet at least several days before use in these experiments.

Diets.—The normal diet was a miscellaneous one consisting of purina fox chow cakes supplemented by wheat, corn, rolled oats, alfalfa hay, lettuce, and carrots. It was believed to be complete and adequate.

*Fisher Scientific Co., No. 21-158.

The low phosphorus diet consisted of the following gelatin 9, egg albumen 9, dextrinized starch and agar (32:1) 60, sodium chloride 1, calcium chloride 1, cod liver oil 1, and butter 10 parts. All constituents were in powder or fine granule form before the cod liver oil and butter were mixed with them. Except for the phosphorus, this diet was considered to be adequate, at least for the period of these experiments. Several of the above ingredients are known to contain phosphorus in small amounts, but the diet as a whole might even be considered to be phosphorus free for most purposes.

Experimental Periods—Rats on the normal diet were bled every three days for a total period of twelve days and the bloods were analyzed. Rats on the low phosphorus diet were bled just before beginning this diet, then every third day for twelve days and on the third and sixth days after resuming the normal diet. Tables I and II indicate the periods, weighings, etc. so that nothing more explanatory need be stated here.

The determination of the effect of chloroform ether anesthesia on the concentration of blood phosphorus compounds is indicated under experimental results.

RESULTS

Changes in Weights of Rats—Considering the rats on the normal diet, it is to be seen in Table I that they lost weight during the twelve days of the experiments. There was an average loss of 17 gm per rat. This was about that of the blood drawn 16.8 cc (18.3 gm).

Table II shows that the rats on the low phosphorus diet lost on the average 26 gm per rat. The average amount of blood drawn (in four bleedings) was 13.4 cc, or 14.7 gm. On resuming the normal diet for six days three of the rats gained an average of 20 gm per rat which was still 9 gm per rat less than the same three rats weighed at the beginning of the experiments.

In order to find what effect the low phosphorus diet had on the weights, we kept two rats on the low phosphorus diet as before, but without drawing any blood, and found the following results:

Rat A	Weight at beginning of experiment	462 gm	Loss, 10 gm
	Weight at end of experiment	452 gm	
Rat B	Weight at beginning of experiment	399 gm	Loss 23 gm
	Weight at end of experiment	376 gm	

Thus the low phosphorus diet as a lone factor caused the rats to lose some weight.

Corpuscle Volume—Table I shows that there was a considerable variation in the red corpuscle volumes of the bloods. On repeated bleedings the regeneration was not in equal proportions in the different rats, on the normal diet there was an average decrease of 29.4 per cent. For the rats on a low phosphorus diet, No. 8 was exceptional in having a low corpuscle volume from the beginning. It decreased little if any on bleeding. The average decrease in corpuscle volume of the five rats was from 43.7 per cent to 34.3 per cent, a decrease of 21.5 per cent. If No. 8 be omitted from the average, the decrease would have

TABLE I

RAT BLOOD ANALYSES. NORMAL DIET. RATS BLED EVERY THREE DAYS

DAY →		0 (START- ING)	THIRD	SIXTH	NINTH	TWELFTH	LOSS
Body weight	RAT NO.	gm.				gm.	gm.
	1	300				282	18
	2	265				250	15
	3	323				308	15
	4	337				317	20
Average		306				289	17
Quantity of blood drawn		c.c.	c.c.	c.c.	c.c.	c.c.	
	1	4.3	3.2	3.8	3.5	3.8	
	2	4.0	3.5	4.0	3.8	3.5	
	3	5.0	4.0	3.9	4.0	3.9	
	4	5.0	5.0	5.0	5.0	3.2	
Average		4.6	3.9	4.2	4.1	3.6	
Hematocrit (red corpuscles)		per cent	per cent	per cent	per cent	per cent	
	1	46.0	43.5	34.0	31.0	30.0	
	2	47.0	43.5	36.0	38.0	37.0	
	3	52.5	44.0	41.0	38.0	36.0	
	4	43.5	36.0	34.0	33.0	30.5	
Average		47.3	41.8	36.3	35.0	33.4	
Plasma inorganic phos- phorus		mg. per 100 c.c.	mg. per 100 c.c.	mg. per 100 c.c.	mg. per 100 c.c.	mg. per 100 c.c.	
	1	5.5	6.2	6.0	6.4	7.9	
	2	6.5	5.7	5.6	5.5	5.6	
	3	6.8	5.8	5.8	5.1	4.7	
	4	4.8	4.9	4.5	4.7	4.3	
Average		5.9	5.6	5.5	5.4	5.6	
Plasma organic acid soluble phosphorus		mg. per 100 c.c.	mg. per 100 c.c.	mg. per 100 c.c.	mg. per 100 c.c.	mg. per 100 c.c.	
	1	2.1	2.2	1.3	0.4	—	
	2	0.3	0.8	1.2	0.5	0.5	
	3	0.3	0.9	1.1	0.7	0.6	
	4	0.5	0.3	0.4	0.5	0.9	
Average		0.8	1.0	1.0	0.5	0.7	
Plasma lipid phos- phorus		mg. per 100 c.c.	mg. per 100 c.c.	mg. per 100 c.c.	mg. per 100 c.c.	mg. per 100 c.c.	
	1	5.2	4.8	6.2	4.5	4.7	
	2	3.8	4.2	5.0	3.8	4.0	
	3	6.4	4.9	4.4	3.9	2.5	
	4	3.6	4.4	3.7	3.3	3.0	
Average		4.7	4.6	4.8	3.9	3.6	
Corpuscle inorganic phosphorus		mg. per 100 c.c.	mg. per 100 c.c.	mg. per 100 c.c.	mg. per 100 c.c.	mg. per 100 c.c.	
	1	3.3	4.4	3.1	3.6	5.4	
	2	5.2	4.7	6.4	5.7	5.3	
	3	5.4	4.5	5.2	5.6	5.4	
	4	3.4	3.9	4.6	4.4	6.2	
Average		4.3	4.4	4.8	4.8	5.5	
Corpuscle organic acid soluble phosphorus		mg. per 100 c.c.	mg. per 100 c.c.	mg. per 100 c.c.	mg. per 100 c.c.	mg. per 100 c.c.	
	1	53.0	—	53.9	60.1	52.6	
	2	67.8	51.3	54.6	—	51.9	
	3	46.7	47.4	55.3	45.9	52.1	
	4	60.8	60.4	57.9	57.0	60.8	
Average		57.1	53.0	55.4	54.3	54.3	
Corpuscle lipid phos- phorus		mg. per 100 c.c.	mg. per 100 c.c.	mg. per 100 c.c.	mg. per 100 c.c.	mg. per 100 c.c.	
	1	9.3	16.6	16.0	17.3	17.9	
	2	11.5	17.9	16.8	14.5	17.7	
	3	19.8	16.9	13.0	17.2	19.0	
	4	16.6	21.2	21.9	18.1	20.4	
Average		14.3	18.1	16.9	16.8	18.7	

been 25.2 per cent. This is still appreciably less than the 29.4 per cent of the rats bled on a normal diet. Resumption of the normal diet showed that a loss was still going on for three days. Then, however, a gain was noted.

Plasma Inorganic Phosphorus—In three rats on normal diets there were definite decreases in the plasma inorganic phosphorus and in one there was a definite increase. The average was a slight and perhaps insignificant decrease of from 5.9 mg to 5.6 mg per 100 c.c., or .45 per cent.

The rats on the low phosphorus diet all showed definite decreases in the plasma inorganic phosphorus, three of these were very considerable. The average decrease was from 5.8 mg to 3.5 mg per 100 c.c., or 38.7 per cent. There was rapid recovery on resuming the normal diet.

Corpuscle Inorganic Phosphorus—On the normal diet two rats' bloods showed no change in the inorganic phosphorus. In two there were increases of 64 per cent and 80 per cent. The average increase was from 4.4 mg to 5.6 mg per 100 c.c., or 28 per cent.

On the low phosphorus diet two rats' bloods were exceptional in that the corpuscle inorganic phosphorus was already low at the beginning. These did not decrease on bleeding, but the corpuscles of the other two rats decreased in inorganic phosphorus. In one rat where the value was high at the beginning, there was a decrease from 8.3 mg to 2.5 mg per 100 c.c., or 69.6 per cent. The average decrease was 30.6 per cent.

Plasma Organic Acid Soluble Phosphorus—This fraction is usually small and since the values are obtained by difference no significance can be placed on the variations. It is to be noted in Tables I and II, however, that this fraction is considerably greater than that which is found for human blood. On the low phosphorus diet as well as on the normal diet, the figures for organic acid-soluble phosphorus are too variable to interpret any change.

Corpuscle Organic Acid Soluble Phosphorus—As in human blood this fraction represents by far the largest percentage of the total blood phosphorus. On the normal diet no change can be concluded to be found. On the low phosphorus diet there was a definite average decrease from 59.0 mg to 38.0 mg per 100 c.c., or 35.5 per cent. The normal values were recovered within three days after resuming the normal diet.

Plasma Lipid Phosphorus—On the normal diet the changes were variable and showed no definite trend except Rat 3 which showed a decrease from 6.4 mg to 2.5 mg per 100 c.c., or 61 per cent. If the average were taken, there was a decrease from 4.7 mg to 3.6 mg per 100 c.c., or 24.4 per cent. On account of the individual variations shown, perhaps no conclusions as to the decrease should be made.

On the low phosphorus diet, a small but real decrease from 3.8 mg to 3.4 mg per 100 c.c., or 10.8 per cent, was shown. On resuming the normal diet the original level was recovered within three to six days.

Corpuscle Lipid Phosphorus—On the normal diet there was a definite increase in the corpuscle lipid phosphorus in three out of four rats' bloods. The average increase was from 14.3 mg to 18.7 mg per 100 c.c., or 31 per cent.

TABLE II

RAT BLOOD ANALYSES. LOW PHOSPHORUS DIET, FOLLOWED BY NORMAL DIET.
RATS BLED EVERY THREE DAYS

DAY →		NORMAL DIET	LOW PHOSPHORUS DIET				NORMAL DIET	
		0 (START- ING)	3RD	6TH	9TH	12TH	15TH	18TH
Body weight	RAT NO.	gm.				gm.		gm.
	5	332				303		
	6	234				220		
	7	219				193		214
	8	246				205*		237
	9	286				265		271
Average		263				237		241
Quantity of blood drawn		c.c.	c.c.	c.c.	c.c.	c.c.	c.c.	c.c.
	5	3.8	4.0	3.8	3.5	3.9		
	6	4.5	4.0	3.5	3.0	3.5		
	7	3.5	2.5	2.5	2.5	2.5	2.5	2.5
	8	2.3	2.8	2.8	1.8	2.0	2.5	2.8
	9	4.0	4.0	4.0	4.2	4.5	4.3	4.2
Average		3.6	3.5	3.3	3.0	3.3	3.1	3.1
Hematocrit (red cor- puscles)		per cent	per cent	per cent	per cent	per cent	per cent	per cent
	5	50.0	47.0	43.5	40.0	39.5		
	6	49.0	39.0	36.5	35.0	30.0		
	7	41.5	36.0	34.5	35.0	32.5	29.5	29.0
	8	34.0	33.0	34.0	31.0	33.5	33.0	36.0
	9	44.0	41.5	37.0	35.5	36.0	31.0	35.0
Average		43.7	39.6	37.1	35.3	34.3	31.1	33.3
Plasma inorganic phosphorus		mg. per 100 c.c.	mg. per 100 c.c.	mg. per 100 c.c.	mg. per 100 c.c.	mg. per 100 c.c.	mg. per 100 c.c.	mg. per 100 c.c.
	5	4.9	4.4	4.9	4.9	4.2		
	6	5.6	5.3	3.8	3.7	4.7		
	7	7.6	5.9	4.7	4.8	4.4	5.5	5.7
	8	5.4	5.6	3.2	2.4	1.8	2.6	4.1
	9	5.3	2.2	2.2	2.9	2.6	6.9	5.6
Average		5.8	4.7	3.8	3.7	3.5	5.0	5.1
Plasma organic acid soluble phosphorus		mg. per 100 c.c.	mg. per 100 c.c.	mg. per 100 c.c.	mg. per 100 c.c.	mg. per 100 c.c.	mg. per 100 c.c.	mg. per 100 c.c.
	5	0.5	0.8	0.9	0.4	0.9		
	6	0.1	1.6	0.9	0.9	1.0		
	7	0.2	0.2	0.6	1.0	0.3	0.3	0.7
	8	0.7	0.6	1.7	0.9	2.4	2.9	1.5
	9	0.9	—	0.6	—	0.7	0.6	0.7
Average		0.5	0.8	0.9	0.8	1.1	1.3	1.0
Plasma lipid phosphorus		mg. per 100 c.c.	mg. per 100 c.c.	mg. per 100 c.c.	mg. per 100 c.c.	mg. per 100 c.c.	mg. per 100 c.c.	mg. per 100 c.c.
	5	4.6	4.1	3.2	3.7	4.2		
	6	2.5	3.1	3.7	3.8	3.3		
	7	3.7	3.4	3.5	3.5	2.6	3.9	3.7
	8	3.3	3.3	3.5	2.8	3.7	3.0	3.8
	9	3.7	3.9	3.5	2.9	3.0	3.3	3.9
Average		3.8	3.6	3.5	3.3	3.4	3.4	3.8
Corpuscle inorganic phosphorus		mg. per 100 c.c.	mg. per 100 c.c.	mg. per 100 c.c.	mg. per 100 c.c.	mg. per 100 c.c.	mg. per 100 c.c.	mg. per 100 c.c.
	5	4.7	4.0	3.8	3.5	2.8		
	6	8.3	4.7	3.8	3.3	2.5		
	7	2.2	3.2	2.9	3.3	3.6	5.1	4.4
	8	2.8	3.3	3.7	—	—	4.4	4.2
	9	3.5	2.7	2.6	3.1	3.0	5.2	5.7
Average		4.3	3.6	3.4	3.3	3.0	4.9	4.8

*Average for last three rats = 221 gm.

TABLE II—CONT'D

DAY →		NORMAL DIET	LOW PHOSPHORUS DIET				NORMAL DIET	
		0 (STARTING)	5TH	6TH	9TH	12TH	15TH	18TH
	LAT NO	mg per 100 cc	mg per 100 cc	mg per 100 cc	mg per 100 cc	mg per 100 cc	mg per 100 cc	mg per 100 cc
Corpuscle organic acid soluble phosphorus	5	60.8	52.2	—	52.7	49.2	—	—
	6	56.7	58.3	49.2	45.7	45.0	—	—
	7	57.3	39.3	40.1	52.7	38.4	36.4	36.6
	8	59.0	61.7	61.8	—	—	58.1	60.8
	9	61.0	71.8	50.6	53.1	57.6	75.1	74.6
	Average	59.0	57.5	50.4	51.0	47.5	56.5	57.3
Corpuscle lipid phosphorus		mg per 100 cc	mg per 100 cc	mg per 100 cc	mg per 100 cc	mg per 100 cc	mg per 100 cc	mg per 100 cc
	5	20.6	18.7	14.9	10.9	13.1	—	—
	6	18.1	17.2	17.1	12.4	11.7	—	—
	7	11.5	11.8	9.1	12.6	9.1	12.7	11.9
	8	10.2	18.8	16.4	17.6	21.8	16.8	20.8
	9	19.6	18.6	18.7	17.9	17.9	19.9	22.4
	Average	17.8	17.0	15.2	14.3	14.7	16.5	18.4

On the low phosphorus diet there were decreases in four out of five rats' bloods. The average decrease was from 17.8 mg to 14.7 mg per 100 cc, or 17.4 per cent. On resuming the normal diet the original level was recovered in six days.

Effect of Anesthesia—We have not thoroughly investigated the effect of chloroform ether anesthesia, but the results which we obtained for two rats from each of which we took blood with and without anesthesia, did not vary more than was usual in phosphorus values. Deep and prolonged anesthesia, however, could be expected to cause a considerable change. Bolliger⁶ found a decrease in the plasma inorganic phosphorus from a value of 4.0 mg to a value of 1.0 mg per 100 cc in dogs one hour after an hour of deep anesthesia. The results of others, which he cites, are not in agreement. A comparison of published results from different sources indicates that no account was taken of the effect of short period anesthesia.

On the other hand, we found a striking increase in the phosphorus compounds, both in the plasma and in the corpuscles, when the rats were much frightened and showed fear just previous to the blood drawing.

All of the bloods disclosed some hemolysis, varying from a trace in most of them to somewhat more in several of them. We did not exclude any bloods on this account.

While the low phosphorus food was not well liked by the rats, they did not lose their appetites and ate well.

DISCUSSION

Table III presents a synopsis of the average changes in blood phosphorus levels recorded in Tables I and II. It was constructed in order to show more readily the gross changes that took place in the bloods.

Bloods Obtained Under the Normal Diet—The plasma average showed no change, or, possibly, a decrease in the phosphorus compounds. In the corpuscles there is shown definite increase in the inorganic and the lipid phosphorus. The

TABLE III

SYNOPSIS OF BLOOD PHOSPHORUS CHANGES PRESENTED IN TABLES I AND II

	RATS BLED UNDER NORMAL DIET		RATS BLED UNDER LOW PHOSPHORUS DIET	
	PLASMA	CORPUSCLES	PLASMA	CORPUSCLES
Inorganic phosphorus	Slight decrease (?)	+28.0%	-38.7%	-30.6%
Organic acid-soluble phosphorus	No change (?)	No change	No change (?)	-19.5%
Lipid phosphorus	Slight decrease (?)	+30.8%	-10.8%	-17.4%

inorganic phosphorus cannot be pictured as a source for the lipid phosphorus when these figures, and also the subsequent ones considered in relation to bleeding under a low phosphorus diet, are considered; and there is no evident transformation of phospholipid into inorganic phosphate.

In reference to the corpuscles it is to be noted that the inorganic and the lipid phosphorus increase rather definitely. While this is not true for two of the rats (Table I) whose values were the same at the end as at the beginning of the experiments, it is true for the corpuscles of the other two rats. The average increase for the four rats was 28 per cent.

Although there are fluctuations in the different bloods of the same rats (Table I), it is believed that the average represents a true upward trend, and this conclusion is strengthened when we see that there was the same tendency for the lipid phosphorus of the corpuscles. The reason for these increases is not clear, but it is possible that it may be due to the influx of new red cells having a higher content of inorganic and lipid phosphorus.

Bloods Obtained Under Low-Phosphorus Diet.—The inadequacy of the phosphorus in the diet is reflected by a very considerable decrease in the blood phosphorus compounds. No evident reciprocal quantitative relationship was revealed among them, however. The changes which occurred were considerably different from those which took place in the blood of the rats on a normal diet. The decreases of inorganic phosphate in the plasma and corpuscles, 38.7 per cent and 30.6 per cent, respectively, were very considerable. The decrease of 19.5 per cent in the corpuscle organic acid-soluble phosphorus, while not so significant relatively, was, however, significant. It is thought by some biologic chemists that the substances from which this phosphorus (sometimes called "ester phosphorus") is obtained, functions more in the nature of food material than the other fractions. Evidently this class of phosphorus compounds is not so much depleted as some of the others studied.

The low phosphorus diet caused the rats to lose more weight than did those on the normal; the latter lost only practically the same as the total weight of the blood which was taken from them, an average of 1 c.c. to 2 c.c. of blood each day. That the dislike for the low phosphorus diet was appreciable is clear, since two rats which were kept on this diet but from which no blood was removed every three days, lost appreciably in body weight. The results obtained are presented under "results." In general the consumption of the low phosphorus diet was observed to be good, though not as good as the normal. The work of other investigators indicates that the phosphorus of the food is a predominating fac-

for Kramer and Howland, for example, when working with growing rats found that "a marked improvement in weight follows the addition of phosphorus to the diet, other factors remaining constant"

It can be calculated from the results of our experiments on the low phosphorus diet, that if one fourth to one third of the total blood of the rats was removed at each bleeding, a total of approximately 75 or 80 per cent of the original blood must have been withdrawn. The loss of inorganic, organic acid-soluble and lipid phosphorus of the blood, however, was less than 40 per cent. Thus there appears to be no question but that other tissues furnished phosphorus to the blood. All three classes of phosphorus compounds which were determined were partially replenished in the blood. A discussion of the possible immediate sources will not be undertaken here.

When it is considered that some phosphorus must have been excreted in the urine and feces during the experiments on the low phosphorus diet, the replenishment from extravascular sources must have been greater than at first appeared.

When we consider, in a general way, the fluctuations of the three classes of phosphorus compounds in the blood it is evident that the food phosphorus is readily utilized as inorganic phosphorus and readily forms phospholipids and organic acid soluble phosphorus substances. When this source of phosphorus fails in the animal, there is only a limited endogenous supply and as a consequence the plasma and corpuscle phosphorus decrease. This indicates that a constant supply of phosphorus in the food is necessary in order to maintain the normal levels in the blood. How much if any, damage results to an animal if phosphorus is inadequate for short periods is difficult to determine. It may be of considerable importance as a factor in the etiology of certain body conditions or diseases, for example, in dental caries.

SUMMARY AND CONCLUSIONS

A technique for repeated withdrawal of one fourth to one third of the total blood of rats is described. This technique was applied to rats kept on normal and on low phosphorus diets. The bloods were analyzed by microchemical methods for the effects of such blood withdrawals on the levels and distributions of the phosphorus compounds.

On the normal diet the rats recovered only a little of the weight which was lost by blood withdrawal. They became anemic, as indicated by the decline in the corpuscle volume. The levels of the phosphorus compounds were not much changed although considerable individual variations in both plasma and corpuscles occurred.

On the low phosphorus diet the rats lost considerably more weight than the weight of the blood which was withdrawn. They became anemic. There were very marked reductions in the levels of most of the phosphorus compounds of both plasma and corpuscles. Recovery of the levels was rapid when the rats were returned to the normal diet.

It was evident from the results on the low phosphorus diet that some phosphorus was transferred from the tissues to the blood.

It is concluded that rats utilize food phosphorus for blood inorganic, organic acid soluble, and lipid phosphorus compounds very readily, but that when the food source fails there is only a limited endogenous supply and as a consequence the plasma and corpuscles become depleted in this element.

There is little relationship between the classes of blood phosphorus compounds; the ready conversion of one into another was not evident in these experiments. A constant supply of phosphorus in the food is necessary in order to maintain the normal levels of phosphorus compounds in the blood. How much, if any, damage results to an animal if this element is inadequate in the diet for short periods is difficult to determine.

REFERENCES

1. McGowan, J. P.: Further Investigations Into the Nature of Vitamin D Action, *Biochem. J.* 27: 943, 1933.
2. Muller, G. L., and Heath, C. W.: Cholesterol and Lecithin Phosphorus in Plasma of Anemia Other Than Pernicious Anemia, *Arch. Int. Med.* 52: 288, 1933.
3. Youngburg, Guy E., and Youngburg, Mamie V.: The Distribution of Phosphorus in Normal and Cancer Bloods, *J. LAB. & CLIN. MED.* 16: 253, 1930.
4. Heymann, Walter: Untersuchungen über die Phosphatstoffwechselstörung bei Rachitis, *Ztschr. f. Kinderh.* 51: 673, 1931.
5. Youngburg, Guy E., and Youngburg, Mamie V.: A System of Blood Phosphorus Analysis, *J. LAB. & CLIN. MED.* 16: 158, 1930.
6. Bolliger, Adolph: Phosphate Metabolism as Related to Anesthesia, *J. Biol. Chem.* 69: 721, 1926.
7. Kramer, B., and Howland, J.: Factors Which Determine the Concentration of Calcium and of Inorganic Phosphorus in the Blood Serum of Rats, *J. Nutrition* 5: 39, 1932.

METHODS FOR DETERMINING ERYTHROCYTE PERMEABILITY*

JOHN KOOPMAN, AND I. DAVID FALKER, NEW YORK, N. Y.

IN A STUDY of osmosis to determine whether partial hemolysis in hypotonic solutions is due to complete hemolysis of some of the cells, or to partial hemolysis of all the cells, several rapid tests were developed for determining the permeability of erythrocytes to any substance that does not react with them.

Inasmuch as the evidence of penetration of substance into erythrocytes is the production of hemolysis, a consideration of the theoretical background is essential for a proper understanding of the fundamental reactions which take place in these tests.

The nature of the reaction which takes place when cells hemolyze in water has been a subject of speculation for many years. One explanation of hemolysis in the presence of water is that,⁴ "If the osmotic pressure of the plasma is lowered by dilution, the pressure within the corpuscle remains high, and water is absorbed by the cell. If this absorption is sufficient, the cell ruptures and the hemoglobin is discharged."

*From the Wassermann Laboratory of the Department of Health.
Received for publication, July 23, 1935.

This explanation does not suffice when the phenomenon of partial hemolysis is encountered. A hypotonic solution added to a suspension of erythrocytes may not cause complete hemolysis. The cells that have not been hemolyzed cloud the solution, and finally settle out. The question which arises is, if the hemolytic agent, i.e., the difference in osmotic pressure, is sufficient to hemolyze some of the cells, why is it not sufficient to hemolyze all the cells?

One explanation which has been offered to account for partial hemolysis is that some cells are more resistant than others. The same kind of incomplete reaction is obtained when a number of bacteria are subjected to the action of a disinfectant. Some of the organisms are killed, others are not.

Airhenius¹ investigated both problems and his conclusions are as follows: "There is no doubt that cells in a sample of bacteria or red corpuscles possess a different power of resistance to a deleterious substance." Brooks³ writes similarly: "In the case of hemolysis as well as in the case of disinfection it is not the fundamental reaction but the difference in individual resistance that determines the course of the process." Parpart⁵ and Saslow⁶ are of the same opinion. Baron,² however, disagrees with this all or none idea and believes that hemolysis is a partial process, i.e., all the cells may lose some hemoglobin.

Those who believe that hemolysis is a partial process must demonstrate that cells do not vary in their resistance and also must explain why the cells remaining become fewer in number as partial hemolysis progresses. The fact that they do become fewer has been demonstrated by Parpart.⁵

The material presented here leads us to think that hemolysis is an all or none phenomenon and demonstrates that cells in hypotonic solutions do not hemolyze because of the absorption of water into the cells, but rather because of the pressure of the electrolytes within the cells. It is for this reason that it was possible to devise the tests outlined here for erythrocyte permeability.

The erythrocyte solutions used were 5 per cent suspensions of washed sheep cells in 0.85 per cent saline.

When 1 c.c. of a 10 per cent solution of urea in water is added to 0.1 c.c. of these cells, they hemolyze immediately. A 10 per cent solution of urea has an osmotic pressure in excess of physiologic salt solution but this excess pressure does not keep the cells from hemolyzing. It cannot be said that the cells hemolyze because the urea harms them in any way for when the same percentage of urea is added to physiologic salt solution the cells will remain intact in this solution just as long as they do in the ordinary normal saline. However, after standing a few minutes in this urea salt solution, the cells will hemolyze immediately if they are suddenly subjected to further addition of a large amount of normal saline.

Some important facts will be disclosed if this phenomenon is examined quantitatively. Two tenths cubic centimeter of the cell suspension is added to 0.5 c.c. of the 10 per cent solution of urea in 0.85 per cent saline. When 3 c.c. of normal saline (without urea) are added immediately, i.e., within a second or two, no hemolysis takes place. When a minute or two is allowed to elapse before the 3 c.c. of saline are swiftly added, the salt solution will cause hemolysis to take

place at once. When the urea solution has been in contact with the cells for several minutes, the rapid addition of 2 c.c. of normal saline will cause partial hemolysis. When the normal saline is added 0.5 c.c. at a time to the urea saline cell suspension with several minutes between each addition, no hemolysis will occur even after 3 c.c. have been added.

An explanation of what takes place under these circumstances is the following:

When a water solution of urea is added to the cells, the urea enters the cells rapidly and the osmotic pressure of the urea within the cell soon equals the pressure of the urea in the outside solution. The pressure of the electrolytes normally contained in the cells is therefore not balanced by any outside pressure and the cells hemolyze just as they would in water.

When the urea is put in normal physiologic saline, the circumstances are somewhat different. The urea enters the cell and again equalizes itself between the cell and the outside solution. No hemolysis takes place because the salts within the cells are balanced by the 0.85 per cent salt solution. If the cells are now overwhelmed by the sudden addition of physiologic saline, the outside urea solution is very rapidly reduced in urea concentration. The effort of the urea within the cell to restore osmotic equilibrium is sufficient to burst the walls and cause hemolysis.

In the first instance where the 10 per cent urea in water was added to the cells, the hemolysis was caused by the pressure of the electrolytes normally present within the cells and not by the urea. In the second instance where the 10 per cent urea in 0.85 per cent saline was used, the hemolysis was not caused by the electrolytes normally within the cells but by the pressure of the urea.

Whenever the salt solution is added to the cells before the urea has had time to enter, no hemolysis takes place because decreasing the urea concentration of the outside solution does not change the internal pressure. Should 3 c.c. of normal saline be added slowly to the cells in the urea saline solution, the urea can leave the cells slowly, and its pressure is not sufficient to burst the cell walls. When the urea is taken out of the cells more rapidly, the pressure may burst the walls of some cells and cause the resultant hemolysis.

There are many substances which will act in the same manner toward red cells. They will penetrate the cell and upon removal from the cell they burst it. In general it may be said that whether or not they cause hemolysis when they leave the cell depends upon the rapidity with which they are removed from the cell as compared with the time it takes for them to enter.

Glycerin shows this phenomenon to the best advantage. There is no immediate hemolysis when a 3 per cent solution of glycerin in water is added to the cells, for the osmotic pressure of the glycerin is greater than the osmotic pressure of the electrolytes within the cells. After the glycerin has entered the cells and has equalized itself so that the cell content of glycerin is equal to that of the external solution, there is no counterbalanced pressure for the electrolyte pressure in the cells and the cells hemolyze. When 3 per cent glycerin in water is added to a cell suspension, it will be about five minutes before the cells will hemolyze. It takes that time for the glycerin to enter the cells.

Cells suspended in a 3 per cent solution of glycerin in normal saline do not hemolyze any more than cells kept in plain physiologic saline. If the cells in the glycerin saline solution are treated in the same way as the cells in the urea saline were treated, it will be seen that when a large amount of normal saline is added, no hemolysis will take place unless the glycerin saline has been in contact with the cells from five to ten minutes. This is much longer than is necessary with cells in the urea saline. The reason for this is that it takes much longer for the glycerin to enter the cells. After a sufficient time has been allowed for the glycerin to enter the cells, the cells hemolyze immediately upon the addition of normal saline. The relative permeability of the cell membranes to various substances can be easily determined by this method.

When the salt solution is added slowly to cells in a glycerin saline solution in order to permit the glycerin to leave the cells without causing hemolysis, the saline has to be added much more slowly than is necessary for the withdrawal of the urea from the cells. The glycerin cannot leave the cells any more rapidly than it enters without rupturing them. For that reason, unless the glycerin concentration outside the cells is reduced very slowly, the pressure of the glycerin within the cells will cause the rupture of the cell membranes and hemolysis.

If erythrocytes were impermeable to glycerin they would not have hemolyzed in the 3 per cent solution of glycerin in water for the osmotic pressure of this glycerin solution is sufficient to counterbalance the pressure of the electrolytes in the cells. When the molar weight of any solute in water equals or exceeds the molar weight of sodium chloride of normal physiologic saline, the osmotic pressure of such solutes is sufficient to prevent hemolysis, excepting in those instances where erythrocytes are permeable to the solutes. In these instances the rate of hemolysis is directly proportional to the permeability of the erythrocytes to these solutes.

A third substance which enters the cell still more slowly than glycerin is sodium chloride. Wherever cells are suspended in saline of double physiologic strength, i.e., 1.70 per cent, it will be found that even after twenty four hours very little salt has entered the cells. When such cells are centrifuged, and the supernatant solution discarded, and a large amount of 0.85 per cent salt solution is suddenly poured on them, no hemolysis takes place. The internal pressure of the cells was not increased by the 1.75 per cent salt solution even after twenty four hours' contact.

The glycerin experiment shows that the slower a substance enters the cell the slower it must be taken out if hemolysis is to be avoided. Inasmuch as sodium chloride enters so slowly, it is difficult to remove it slowly enough to prevent hemolysis.

The problem of removing salts from cells without rupture of the cell was solved by the use of 7 per cent sucrose. After the exposure of cells to a solution of 7 per cent sucrose in water, they show an increased resistance to hemolysis in hypotonic salt solutions. Equal parts of whole blood and 7 per cent sucrose in water were allowed to remain in contact at ice box temperature for twenty four

hours. The tubes were centrifuged and the supernatant fluid was removed. This was replaced with fresh sucrose solution. This operation was continued for ten days and at the end of this time the cells had lost practically all of their salt. *As a result of this procedure, erythrocytes could be added directly to water without undergoing the least amount of hemolysis.* This is the first time this has been accomplished.

As the process of removing the salts from the cells progressed, the successive sugar solutions were titrated for chlorides. Each day the amount of chlorides taken out of the cells by the sugar solution decreased until it was impossible to detect any. On each succeeding day it was also possible to suspend the cells in a sugar or salt solution of lower tonicity without producing any hemolysis.

This experiment with sucrose explains why cells used for complement fixation tests remain intact over long periods of time when the whole blood is preserved with sucrose. It is not because the sucrose acts as a preservative, but because the sucrose lowers the tonicity of the cells by the removal of salts.

The fact that salt free cells may be suspended in water without undergoing any hemolysis confirms the opinion that hemoglobin alone exerts no osmotic pressure. The reason for the hemoglobin leaving the cells is therefore not due to a seepage through the membrane caused by osmotic forces, but because the cell walls have been ruptured. It is also highly problematical whether a molecule as large as hemoglobin can leave an erythrocyte without rupturing its cell wall.

The reason that salts like sodium chloride can be removed from cells in sucrose solutions without causing hemolysis is that the pressure of the sucrose upon the cells counteracts the pressure of the salts within the cells so that the cell membranes are under no stress. The cell membrane is impermeable to sucrose but permeable to salt. As the salts leave the cells, the saline pressure decreases and less sucrose is necessary to prevent hemolysis.

The speed with which the salts can leave the cells without causing hemolysis is determined by the permeability of the cell membranes to them. These membranes are so relatively impervious to binary salts that it takes approximately ten days to remove the salts from the cells. The reason for the steady decrease in speed with which the salts leave the cells seems to be due to the fact that as the saline concentration of the cells diminishes, the saline pressure declines.

When red cells are kept in contact with 6 per cent sucrose in 0.2 per cent saline, no hemolysis will take place. If every twenty-four hours the cells are centrifuged and the supernatant fluid removed and replaced with fresh sucrose saline solution, in about a week these cells can be suspended in a 0.2 per cent saline solution without any hemolysis because the saline concentration of the cells has been reduced to 0.2 per cent. When these cells are placed in a 0.1 per cent saline solution, partial hemolysis results, i.e., some of the cells hemolyze; in water, hemolysis is complete. From this it is seen that regardless of the saline content of cells, they hemolyze in solutions hypotonic to them. When a solution is sufficiently hypotonic to the cells, they all hemolyze.

It is not so much the amount of substance that leaves the cells, but the difficulty with which it passes through the cell membranes that determines hemolysis. Cells with a saline content of 0.1 per cent hemolyze when placed in

water On the other hand, when cells in a 5 per cent urea in normal saline are taken out of that solution and placed in plain normal saline, they very rapidly lose practically all of the 5 per cent urea they contain, yet they do not hemolyze

$$\text{Hemolysis} = R \left(\frac{\text{Permeability of the cell walls to substances leaving the cells}}{\text{Amount of these substances leaving the cells in a given interval of time}} \right)$$

$R = \text{the individual cell resistance to hemolysis}$

CONCLUSION

An analysis of the experimental material which has been presented here causes us to think that hemolysis is an all or none phenomenon It also demonstrates that cells hemolyze in hypotonic solutions because of the pressure of the electrolytes in the cells rather than the absorption of water by the cells

Several methods for determining erythrocyte permeability to any substance have become apparent as a result of the above experimental work They are

1 The substance to be tested is dissolved in normal physiologic saline This saline solution is added to washed erythrocytes If the cells are permeable to the substance tested, after the substance enters the cells, upon the sudden addition of a large quantity of plain normal physiologic saline, hemolysis will take place Hemolysis will take place only when after the addition of normal saline the difference between the amounts of substance outside and inside the cell causes sufficient pressure within the cells to burst them

2 When the molar weight of any solute in water equals or exceeds the molar weight of sodium chloride of normal physiologic saline, the osmotic pressure of such solutes is adequate to prevent hemolysis, excepting in those instances where erythrocytes are permeable to the solutes In these instances, the rate of hemolysis is directly proportional to the permeability of the erythrocytes

These tests are not applicable to substances which either injure or fix the cells

REFERENCES

- 1 Arrhenius, S Quantitative Laws in Biochemistry, New York, 1915, The Macmillan Co, p 63
- 2 Baron, J Über den Mechanismus der hypotonischen Hämolyse, Arch f d ges Physiol 220 243, 1928
- 3 Brooks, S C Mechanism of Disinfection and Hemolysis J General Physiol 1 61, 1918
- 4 Macleod, J J R, Pearce, R G, Redfield, A C, Taylor N B, and Olmstead, J M D Physiology and Biochemistry in Modern Medicine, St Louis, 1930, The C V Mosby Co, p 90
- 5 Parpart, A K Is Osmotic Hemolysis an All or None Phenomenon? Biol Bull 61 500, 1931
- 6 Saslow, G On Supposed Partial Liberation of Hemoglobin From Mammalian Erythrocyte, Quart J Exper Physiol 19 329, 1929

THE LEUCOPENIC INDEX*

II. CONCERNING THE NATURE OF FOOD SENSITIZATION IN INTRACTABLE ALLERGIC DISEASES

HERBERT J. RINKEL, M.D., KANSAS CITY, MO.

IN 1934 Vaughan¹ published two reports concerning the diagnostic value of the leucocytic response following the ingestion of individual foods and designated the test as the leucopenic index. Subsequently, Zeller,² Gay,³ and myself⁴ reported upon the nature and value of this test in chronic asthma, gastrointestinal allergy, and the allergic diseases, respectively.

While using the leucopenic index as a means of determining food sensitization in a number of patients with the various allergic diseases, several important observations were made. One of these, the more or less complete sensitization to all foods as evidenced by this test, is considered of sufficient importance to be dealt with in some detail.

OBSERVATIONS

If a patient subject to hay fever or asthma is kept on a diet of only those foods producing a negative leucopenic index (postingestive leucocytosis) and is at the same time correctly treated for his inhalant sensitivities, there is usually rapid and, more often than not, practically complete relief of allergic symptoms. In those cases of allergy where food alone may be considered the primary etiologic factor, i.e., hives, headaches, and atopic eczema, one again finds rapid improvement when the diet is arranged upon this same basis.

The observation of these facts in the early use of the leucopenic index stimulated the belief that one might, by testing all food groups, be able to arrange a diet which would favorably affect all cases in which food was an etiologic factor. Accordingly, a number of patients were tested with every food group, or with all they cared to eat. These tests were started in October and finished in December, 1934. Subsequent to this, repeated tests were made with many of the foods, and clinical observations have been maintained in all cases during the following eight months.

In the course of this study a finding of great practical importance was that certain individuals with intractable allergy did not have a negative leucopenic index to any food, or if they did have such a reaction, they were not able to maintain it after the food was eaten repeatedly. This observation is considered significant because of the fact that of the seven intractable asthmatic patients on whom this study was originally begun, poor results were obtained in only those instances where this phenomenon existed.

*Received for publication, August 8, 1935.

The clinical importance and the nature of this finding can best be emphasized in the detailed case records presented herewith

CASE RECORDS

Fig 1 is a composite graph of the leucocytic responses of the eleven patients reported in this paper. These graphs are made by dividing the leucocytic responses into three general groups. The group designated as the negative index consisting of those foods which produced an increase of 500 or more white blood cells, the indeterminate group being those with less than 500 cells' variation from the initial count. The positive leucopenic index includes those foods producing a decrease in the white blood cells of more than five hundred cells. The counts were taken just before eating and twenty, forty, and sixty minutes thereafter, and a composite curve was made for each of the three types of leucocytic responses.

In Table I the essential findings of the eleven cases are presented. Since all recognized therapeutic measures had been employed without good results and these patients were considered intractable allergic individuals, discussion will be limited to the nature of the leucopenic index and its clinical application.

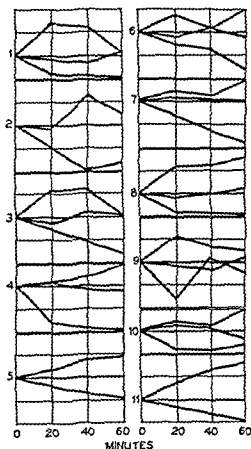


Fig 1—Complete leucocytic responses in eleven patients with intractable allergy. The ordinates represent 1000 cells; the abscissae twenty minutes.

CASE 1—Miss K. E., fifty-six years old, first seen August, 1934. Asthma began following influenza in 1932 and was of such severity as to require between three to eighteen injections of adrenalin daily during the past two years. Six weeks of study was devoid of any real benefit nor had it revealed any etiologic factors except pork and milk.

Leucopenic studies were then made to ten common foods, three of which, wheat, carrots, and eggs were negative. The diet was limited to these foods and on the fourth day of the diet the need for adrenalin ceased. The patient did not use the drug again for two months. At that time a test was made to demonstrate the etiologic importance of pork, and asthma promptly returned. Since then this patient has not had asthma except with new food tests or with known error in her diet. A recheck of her foods in March, 1935, indicated that she had been able to maintain the same type of leucopenic index after using these foods for six months.

The good results obtained in this patient by means of the leucopenic index prompted a similar study of seven intractable asthmatics in whom all ordinary therapeutic measures had been of no avail. This group of patients consisted of Cases 2 to 8 inclusive.

TABLE I

SUMMARY OF IMPORTANT FINDINGS IN ELEVEN CASES OF INTRACTABLE ALLERGY

Case 1 was the original case studied by means of leucopenic indices; cases 2 to 8 inclusive are the unselected group of cases in which good results had not been obtained by regular diagnostic and therapeutic measures during the time indicated. Cases 9 to 11 are additional cases studied by means of the leucopenic index and are included as a means of illustrating the same feature in allergic diseases other than asthma. Good results means the patient was relieved of symptoms by specific food elimination along with proper inhalant therapy (which of itself was ineffective) and that the diet was practical. Fair indicates 75 per cent improvement was recorded by the patient, while poor indicates only slight improvement.

RÉSUMÉ OF FINDINGS	CASES										
	1	2	3	4	5	6	7	8	9	10	11
Asthma	+	+	+	+	+	+	+	+	+		
Urticaria										+	
Migraine											+
Duration of symptoms in years	2	3	5	5	31	20	15	17	7	12	32
Age of patient when first seen	56	6	51	6	33	37	26	41	43	42	37
Months under care before leucopenic indices were used	2	15	16	12	3	10	6	15	3	6	7
<i>Skin Reactions</i>											
Food	0	1	10	0	18	24	11	14	5	27	29
Animal dander	0	3	5	0	4	3	7	6	2	2	0
Pollen	0	4	2	0	3	6	2	3	1	3	0
Number of foods known to increase symptoms before leucopenic indices were made	2	15	24	1	33	6	22	3	2	3	2
<i>Leucopenic Indices</i>											
Number run	17	8	12	11	17	17	30	19	39	18	20
Negative indices	9	4	4	1	2	5	7	4	18	8	7
Indeterminate indices	1	0	3	4	0	1	2	4	5	1	4
Positive indices	7	4	5	6	15	11	21	11	16	9	9
Number of negative indices becoming indeterminate or positive with use of food constantly	0	0	1	0	2	5	7	4	15	8	7
Results of treatment using leucopenic indices to govern diet	Good	Good	Good	Good	Poor	Poor	Poor	Poor	Good	Poor	Fair

CASE. 2.—Miss P. C., aged six, seen in September, 1933, because of asthma of three years' duration, always worse in autumn. During thirteen months' study it was determined that fifteen foods would produce asthma and that she was sensitive to ragweed and grass pollen. The patient had been fairly well relieved until the onset of the grass pollen season; thereafter no improvement was noted in spite of the fact that she was given adequate pollen dosage.

Leucopenic studies were made with seven foods, four of which were negative. The patient was placed upon a diet of these four foods. Within three days asthma ceased and she has not had a single attack during the past ten months except with either a food test or with error in the diet. New foods have been added until today she is able to eat some fifteen foods. She has been able to maintain a negative leucopenic response to the foods which originally agreed with her.

CASE 3.—Mr. C. L., fifty-one years old, came because of perennial asthma of five years' duration. He had been found house-dust sensitive elsewhere. The patient knew a number

of foods which would provoke asthma. In the course of sixteen months' treatment, it had been proved that 24 foods would definitely increase his asthma and that house dust injections were necessary in his treatment. Leucopenic studies were made with twelve foods, four of which were negative. A diet arranged upon this basis produced immediate and complete freedom from symptoms for about one week, then symptoms recurred. A recheck of the leucopenic indices indicated a positive index in the case of one food and with its elimination subsequent good results continued for six months. At that time he took evaporated milk because someone told him it might not cause asthma. Within an hour he was wheezing and required adrenalin at two or three hour intervals for five days for relief of symptoms. This was the only need for adrenalin in eight months following the use of the leucopenic studies.

CASE 4—Miss J A, aged six came because of asthma the year round for five years. She had been treated for a year with indifferent results. Leucopenic indices were made with eleven foods. Since December, 1934, the patient has been without asthma except for four attacks, three of these being with new food tests, the other due to an error in the diet. She has been able to maintain her tolerance for all foods originally negative.

CASE 5—Mrs G R, thirty three years old was seen on account of asthma from which she had suffered since two years of age. There was considerable emphysema and chest deformity. The usual studies were without benefit except to indicate that milk and many other foods would provoke severe asthma. Trial diets were not practical due to the almost constant wheezing. (In a case like this a diary is almost useless.)

Seventeen leucopenic indices were made of which only potato and tomato were negative. She was requested to live on these two foods and during the three weeks that she ate potato and tomato her asthma ceased entirely and she gained six pounds. At that time she was given foods with positive leucopenic indices and asthma recurred. Pork which had produced a positive leucopenic index in November and had been omitted since was then retested and found to give a negative leucopenic index. She was able to eat pork for two weeks then symptoms recurred with eating it.

Thus patient's almost universal sensitization to foods, her ability to remain free of symptoms upon a diet of foods giving negative leucopenic indices, her inability to maintain a tolerance for foods taken constantly in liberal quantities as well as her ability to develop a tolerance to omitted foods are the main points of interest.

CASE 6—Mr C D, aged thirty seven had suffered with perennial asthma for twenty years. The cereals were known to precipitate severe asthma but no other specific cause could be found for his almost constant symptoms. He was dismissed due to our failure to improve his condition and then recalled for leucopenic studies in November, 1934. Tests were made to seventeen foods, five of which were negative. A diet of these foods was given and a recheck was made within a week. It was found that he was unable to maintain a negative leucopenic index to any of these foods. Treatment was therefore discontinued.

CASE 7—Miss P S, twenty six years old, had suffered with perennial asthma for fifteen years. During the course of six months' study it was found that 22 foods would definitely increase her symptoms. She had by the usual therapeutic measures been relieved enough that the adrenalin dosage had been reduced from 6 to 8 per day to 2 or 3. Leucopenic studies were made to thirty foods, seven of which were negative. A diet arranged on this basis was followed by improvement beginning in two days, and continuing for several days, when symptoms recurred again. A recheck to all foods indicated the development of a positive leucopenic index in every case. Treatment in her case was therefore impossible upon the basis of specific elimination.

CASE 8—Mr J H, aged forty one, asthma perennially since 1918. In his case it was definitely determined that cereals would produce asthma, as would potatoes, beans, and pork. Treatment in his case has been most exhaustive, his cooperation the best, but without more benefit than to reduce his attacks from six to eight to one light attack daily.

Nineteen leucopenic indices were made, four being negative. A trial diet was not effective, the leucopenic indices becoming positive within a few days to every food originally negative. In his case it was found that omission tended to give negative indices except with the cereals which were positive after two years' omission.

CASE 9.—Mr. J. H., forty-three years old, asthma perennially seven years. This patient was thoroughly studied by means of the leucopenic indices, a total of 39 tests being made. He was able to maintain a negative leucopenic index for the three months under observation to wheat, cabbage, and tea. The other 13 negative tests became positive after eating the foods. In one case this change occurred within forty-eight hours. His results were only fair due to the limited diet. However, he has gained 18 pounds and is not using adrenalin upon this plan of treatment.

CASE 10.—Miss M. S., aged forty-two, was seen for perennial vasomotor rhinitis and hives of twelve years' duration. Treatment was indicated with house dust and orris root extracts. Leucopenic studies were made with 18 foods. Of the 8 negative tests, none remained so with constant use of the foods. Specific dietary measures being impossible, dietary alternation was begun two months ago. The patient has been practically free of hives during the past two months without medication except desensitization with the inhalants mentioned. Her results had been poor upon the basis of specific elimination, but were fairly good with food alternations.

CASE 11.—Mrs. C. W., thirty-seven years old, had suffered with headaches for thirty-two years which had become practically constant for the past three years. Leucopenic studies were started at once, 20 tests being made. There were seven negative indices. A diet was arranged upon this basis and the foods were rechecked after one month. It was found that all seven of the negative indices had become either indeterminate or positive. Therefore, the diet was limited to the more favorable reactors. There were 186 hours of headache over a ten-day period on a general diet compared to only 46 hours of headache in the same period of time while using a diet arranged with the aid of the leucopenic indices. Milk which had given a positive index was recently taken and produced severe symptoms.

COMMENT

It should be emphasized that in every instance these patients had previously been treated and studied by every recognized therapeutic measure. In all the asthmatics, therapeutic trial had been made with those inhalants giving skin reactions with which the patient was in contact. Isolation in dust-free rooms, autogenous vaccines and gastrointestinal studies likewise had been made where indicated.

The first point of interest is that in a series of seven severe asthmatic patients who could not be relieved by ordinary diagnostic or therapeutic measures, there were three who were promptly cleared up on the basis of the leucopenic studies. This fact is of considerable interest, in that of those relieved all were able to maintain a tolerance (determined by the leucopenic indices) for some foods which were used constantly. In contradistinction to this there were either no negative indices, or if they were originally negative, they soon became positive in those patients in whom the leucopenic indices were of no value.

The second point of interest is the finding that about half of these intractable allergies react unfavorably to nearly every food. Our observation in this series of patients has been similar to that of Zeller.² The question of whether or not the leucopenic responses are specific in those cases where practically all

foods produce a positive leucopenic index has been raised by Vaughan.⁵ This may or may not be true. For the present, one can only report the fact that the condition does exist, and in its presence, there has been therapeutic failure.

In these cases, the inability to maintain tolerance for a food, and the almost universal sensitization to food present an important problem in diagnosis and therapy, and suggest a logical explanation for the cause of these intractable cases. In these patients the protective mechanism has almost completely broken down or become ineffective. That the normal tendency is to recover is indicated by the fact that with the omission of foods giving positive leucopenic indices the reaction tends to become negative again.

These observations have not only enlightened us in the diagnosis of the cause of these intractable cases of allergy but have also suggested more efficient means of treatment.

It is evident that one has not ruled out the presence of food allergy in any given case unless he can demonstrate that the patient has been limited to a diet of foods giving negative leucopenic indices.

Treatment upon the basis of specific elimination is the easiest and the most practical procedure in those patients who have the ability to maintain tolerance for foods taken repeatedly, whereas, in those lacking this ability, dietary alterations, not elimination, are the therapeutic method of choice.

CONCLUSIONS

1 About one half of eleven patients with intractable allergy gave positive or indifferent reactions to practically every food.

2 Patients who give a negative leucopenic index to only a few foods rarely tolerate these foods when used constantly.

3 The prognosis in these severe allergies was quite accurately determined by the patients' ability to maintain tolerance for foods, which in turn was determined by the leucopenic index.

4 These studies present an explanation for some cases of intractable allergy.

5 Specific elimination is not possible in all patients with intractable allergy.

REFERENCES

- 1 Vaughan, Warren T. (a) Food Allergens, III, The Leucopenic Index, Preliminary Report, *J Allergy* 5 601, 1934.
(b) Further Studies on the Leucopenic Index in Food Allergy, *J Allergy* 6 78, 1934.
- 2 Zeller, Michael. The Leucopenic Index in Intractable Asthma, *Ill Med J* 69 54, 1936.
- 3 Gay, Lee Petit. Personal Communication.
- 4 Rinkel, H. J. The Leucopenic Index in Allergic Diseases. Read Before the Thirteenth Annual Meeting of the Association for the Study of Allergy, Atlantic City, June 11, 1935. *J Allergy* 7 356, 1936.
- 5 Vaughan, Warren T. Personal Communication.

SAFELY INDUCED FEVER THERAPY IN A DIABETIC INDIVIDUAL*

B. Y. GLASSBERG, M.D., ST. LOUIS, MO.

IT WOULD appear that many physicians consider diabetes a contraindication to certain forms of therapy designed to ameliorate other coexisting disease syndromes. In particular it seems widely thought that induced fever therapy is likely to result unfavorably. It is our feeling that the modern antidiabetic armamentarium is relatively so perfect that in skilled hands any other coexistent disease may be treated just as if the diabetes did not exist. For that reason the following case report of successfully executed malarial therapy in a diabetic person with paresis may be of interest.

CASE REPORT

M. B., male, aged forty-eight years. The patient first reported to the Aaron Waldheim Health Clinic in April, 1931, with the chief complaints of headache, occasional abdominal pain, and tenesmus. Otherwise the history was essentially unimportant, except that the patient specifically denied venereal infection. Physical examination revealed no important abnormality except a barely palpable spleen. The urine contained much sugar but no albumin or formed elements. The glucose tolerance curve was diabetic in type; the patient was placed upon an antidiabetic régime without insulin. Two months later when he complained of typical girdle pains, a Kahn test, inadvertently omitted in the earlier examination, was found to be three-plus, on repetition, four-plus. He was referred to the St. Louis Municipal Dispensary for antisyphilitic therapy. In October, 1932, the patient presented the picture of an acute toxic (arsenic) hepatitis with jaundice; this cleared up without special treatment and arsenic therapy was cautiously resumed in June, 1933. The diabetes which up to this time had been adequately controlled without insulin, became more severe as manifested by continuous glycosuria and rising blood sugar levels. At the patient's insistence insulin was postponed until March, 1934, when a blood sugar persistently above 150 mg. per cent made its use seem very wise. The next month parietic manifestations appeared and the patient was placed in the Jewish Hospital for study. Blood Wassermann (cholesterinized and noncholesterinized antigens) and Kahn tests were four-plus. The spinal fluid showed a four-plus Wassermann in both antigens and the Kahn test was three-plus; the colloidal gold curve was 1-1-2-3-3-2-1-0-0-0. Dr. Sidney I. Schwab, the neurologic consultant, made a diagnosis of paresis but advised against malarial therapy on account of the diabetes. The patient was therefore discharged from the hospital on essentially the same diet as before with a daily insulin requirement of 35 units. In response to our insistence Dr. Schwab consented to send the patient back into the hospital for malarial therapy.

Accordingly the patient was readmitted into the hospital on Aug. 2, 1934, and placed on a diet of protein 60, fats 100, carbohydrates 150, and 55 units of insulin (divided 20, 15, and 20). Five cubic centimeters of malarial blood were injected intravenously and the following day 8 c.c. were given. On August 8 the first chill occurred with a rise in the rectal temperature to 102° F.; on the following day another chill occurred and the temperature rose to 105.4° F. Thereafter he had chills every second day until August 15; from the fifteenth to the twenty-first he had daily chills with the highest rectal temperature recorded as 106.8° F. After

*From the Diabetic Division of the Aaron Waldheim Health Clinic, Jewish Hospital.
Received for publication, August 8, 1935.

the tenth chill quinine was given to cure the malarial infection. The patient was discharged from the hospital three days thereafter on a diet of P 60, F 150, C 100 with 60 units of insulin (divided 25, 15, and 20) each day.

The diabetes was controlled during this course of treatment by a plan previously described by Glassberg.¹ The original or maintenance insulin requirement was determined in this manner. In addition, it was felt that a definite insulin requirement could be determined in essentially the same manner to combat the hyperglycemia induced by the hyperpyrexia. Twenty units of insulin was given at the time of the first chill and the blood sugar two hours later was 171 mg per cent. Accordingly the insulin at the time of the next chill was increased to 25 units and because the blood sugar was 182 mg per cent two hours later, the "chill" insulin dose was increased to 45 units. In view of a rising blood sugar level the daily maintenance insulin dose was increased to 75 units (divided 25, 15, 20, and 15, the last at 8 P.M.). On this regime the patient's diabetes was adequately controlled as evidenced by the fact that of 10 blood sugar determinations made at various times only one was above 200 mg per cent (222), the rest varied between 108 and 188 mg per cent. There were no hypoglycemic reactions. While glycosuria was a not infrequent occurrence no attention was paid to it in view of the better control possible from blood sugar observations. On the regime prescribed for the patient at discharge from the hospital the blood sugar two hours after breakfast was 127 mg per cent. The blood Kahn test was still four plus.

The patient continued under observation in the clinic where a steady decrease in the insulin requirement was demonstrated by falling blood sugar levels, seven weeks after discharge from the hospital (seven and one half weeks after the last chill), the insulin requirement was 40 units each day, it has since continued at this level.* When one recalls that the daily insulin requirement before fever therapy was 35 units, it becomes evident that the induced chills had no deleterious effect upon the diabetic process. The parietic manifestations showed some improvement.

DISCUSSION

In an earlier paper¹ I expressed the opinion that the maintenance insulin requirement could be simply determined by the following mathematical formula

$$\frac{\text{Blood sugar (preferably 2 hr after breakfast)}}{100} \times \frac{100}{\text{Body weight in kilos}} + \frac{\text{Available glucose in diet}}{4}$$

To determine the "chill" insulin requirement, the first half of the formula was used except that the blood sugar two hours after the chill is substituted for that two hours after breakfast. As pointed out in that paper, only 80 per cent of the calculated insulin dosage is given in order that a safety factor may be present and until later blood sugar observations show the need of modification of that dosage. While it would be possible to show the successive steps by which the insulin requirement in the case reported was determined, it would seem simpler to present a brief resume of the case and the formula which was used to control adequately the diabetes. It may be added that this formula has given me uniform satisfaction in determining the insulin requirement of many complicated and uncomplicated diabetic problems.

SUMMARY

A case report is presented showing the case with which the diabetes was controlled in a parietic patient given malarial therapy. The opinion is expressed that the present antidiabetic armamentarium should enable the qualified phy-

*Now twenty one months after fever therapy the daily insulin requirement is five units.

sician to treat coexistent complications as if the diabetes did not exist. A formula which the writer has found invaluable for this purpose is presented. It is to be noted that the induction of 10 malarial chills in this patient had no deleterious effect upon the course of the diabetes.

REFERENCE

1. Glassberg, B. Y.: Gauging the Dose of Insulin, *J. LAB. & CLIN. MED.* 19: 1173, 1934. METROPOLITAN BUILDING.

THE SELECTIVE ELIMINATION OF NEUTRAL RED THROUGH THE GASTRIC MUCOSA*

EXPERIMENTAL APPROACH

SAMUEL MORRISON, M.D., RAYMOND E. GARDNER, Sc.D., AND
DAVID L. REEVES, M.D., BALTIMORE, Md.

IN A previous publication¹ we have reported the elimination of a large series of dyes through the gastric mucosa. In that communication the variation in results obtained by other investigators was discussed. In this present work we have chosen neutral red as an exemplary dye with which to demonstrate the selective elimination of dyes through the gastric mucosa, yet not all dyes need necessarily to be eliminated by the same mechanism. Moreover, the staining properties of dyes in fixed tissues may be entirely different from those in living tissues, since vital staining is not fixed tissue staining. At one time we thought there was evidence that basic dyes stained certain cells (parietal or oxyntic), while acid dyes stained other glandular tissues (peptic cells). In another study we thought acid or basic dyes may select either peptic, parietal or mucous cells for selective elimination, but this observation was not borne out in later experiments. The difficulty about the problem is that vital dyes cannot easily be fixed in situ. Also, a cell may be in the process of dying, or even dead, when first taking up the stain. These make a difference in the color of the dye, the reaction of the cell, the depth of staining, and the ability to hold the stain.

It has been seen that Dawson and Ivy² observed several dyes, of which neutral red is the best example, can be detected by microscopic examination to be passing through the parietal cells as the secretion in the canaliculi is rendered conspicuous by the dye; other dyes which are liberated more slowly cannot be detected with certainty in any part of the mucosa. Although the conclusion is reached that the parietal cells are the cells chiefly concerned in the elimination of dyes by the gastric mucosa, it is argued that "in an organ with such diverse histologic structure as the stomach it is difficult to be positive that

*From the Department of Medicine (Gastro-Enterology), University of Maryland, and the Department of Immunology and Department of Surgery, Johns Hopkins University.
Received for publication, August 15, 1935.

all dyes are being eliminated by the same cells. That is, some dyes may possibly be eliminated through the parietal cells, others through the chief cells or mucous cells. Further, the fundic mucosa may not be the only portion of gastric mucosa which participates in the elimination of dyes. The pyloric mucosa may also be active. It has been assumed by several workers that the fact that dyes are not eliminated by a resting mucosa, but pass out through an active one, is sufficient evidence that only the fundic mucosa is involved in this process. Ivy and Oyama (1921) have shown, however, that the normal stimulus for the activation of the pyloric mucosa is gastric juice, especially its acid component. Accordingly in a resting stomach, i.e., one which is not actively secreting acid, the pyloric mucosa may also be inactive. The wide variation in the time required for different dyes to appear in the pouch might therefore be explained on the ground that some dyes are eliminated by one type of epithelial cell and others by a different type of cell."

Kobayashi³ also comes to the conclusion that the elimination of dyestuffs can be explained by the secretory function of the gastric glands, especially of the parietal cells, which manage the secretion of hydrochloric acid. He was able to show that when the parietal cells secrete HCl in a high concentration, they secrete dyestuffs in a relatively large quantity, there was a parallelism between the two which was not true of pepsin. There was a tendency for the speed of decrease in the concentration of dyestuffs to occur more rapidly than that of the acid but this was due to the fact that the dyestuffs were eliminated from the liver, the kidneys and other organs, and the concentration of them in the blood decreased rapidly.

Henning,⁴ by direct microscopic study, concluded that dyestuff excretion is a function of the fundus glands, and that the antrum glands do not participate. He finds that the excretory function of the gastric mucosa is connected with the secretory function, since it can be elicited in secretory rest, by means of secretory stimulants (histamine).

In our work we were actually able to fix the neutral red in the parietal cells, something which had not been done before. The stain was definitely confined to the oxyntic, parietal, or acid secreting cells. It is interesting that Dodds, Noble and Smith⁵ have found that the posterior lobe of the pituitary contains a substance capable of inducing a severe lesion of the acid bearing area of the stomach. If the direct toxic action on the cells of the acid secreting area of the stomach became general an important experiment could be performed with neutral red for under such conditions neutral red should not be eliminated. This would add another convincing experiment to prove the specific elimination of neutral red.

The steps which lead to our success in fixing neutral red in the parietal cells are important to relate. Since the stomach is acid in reaction, it was decided to bring an aqueous or saline solution of neutral red in contact with hydrochloric acid and then to add various other agents. When precipitates formed, that combination was considered a possible means of fixing neutral red in the gastric wall for most of the agents were really fixing agents. At the same time the effect of excess HCl, distilled water and ethyl alcohol were determined. In this manner it was found that 3 per cent K_2CrO_4 , saturated picric acid, 5 per cent

and 10 per cent ammonium molybdate, 2 per cent aqueous solution of potassium ferrocyanide, 1 per cent phosphotungstic acid, Zenker's solution, KMnO_4 (1 per cent), Bouin's solution, phosphomolybdic acid, bichloride of mercury, saturated solution of chromic acid, acid fuchsin (1 per cent) and Lugol's solution all produced precipitates unaffected by excess HCl , distilled water or ethyl alcohol. Ferric ammonium sulphate (10 per cent), formalin (40 per cent), gentian violet, glacial acetic acid, potassium ferrocyanide (2 per cent), trichloroacetic acid (1 per cent), and sodium phosphate (10 per cent) produce no precipitates when subjected to the same reaction. Neutral red (1 per cent) in the presence of a saturated solution of bicarbonate yields a reddish precipitate which remains when distilled water, ethyl alcohol and methyl alcohol are added to separate portions but disappears when HCl is added to excess. Light green gives a faint precipitate with 1 per cent neutral red which is hardly affected by distilled water, 1 per cent HCl and methyl alcohol but is diminished by addition of ethyl alcohol. Xylol and acetone were also tried for their effects on these different reactions because they are used in the process of fixing tissues, and except in the soda bicarbonate reaction (in which the precipitate dissolves) the reactions are essentially the same as with water and the alcohols. Chloroform did not mix with 1 per cent neutral red (aqueous solution). Certain complicating reactions occurred such as the solubility of the precipitates formed with Lugol's, saturated picric acid and potassium bichloride in alcohol on standing and the production of thin thready crystals by neutral red added to tap water after a few hours; the latter change does not occur when distilled water is used.

After these preliminary observations 40 c.c. of 1 per cent neutral red were given intravenously to a dog ($12\frac{1}{4}$ pounds) and as the dye came through the stomach (about one hour seemed the opportune time in most animals) blocks of tissue were taken and immediately placed in the various fixing solutions (3 per cent K. bichromate, 1 per cent phosphotungstic acid, Bouin's, sat. solution chromic acid, Zenker's, formalin, a phosphomolybdic-formic-acetic acid formula, 1 per cent phosphomolybdic acid, solution A B, Zenker's with 15 per cent formalin (fresh), saturated picric, Zenker-formalin (old) HgCl_2 , and in the following nonfixing solutions (1-sat. sol. soda bicarbonate, 2-2 per cent K ferrocyanide, 3-Lugol's, 4-acid fuchsin 1 per cent, and 5-Am. molybdate 5 per cent). These latter were then placed in absolute alcohol (1, 2, 3, 4, 5), mercuric bichloride (1, 2, 4), phosphotungstic acid (1, 2, 3, 4, 5), phosphomolybdic acid (1, 2, 3), and the phosphomolybdic-formic-acetic acid formula (4, 5). Both these groups were carried through to paraffin. The color (of neutral red) remained only in the mercuric bichloride, phosphomolybdic acid 1 per cent, Solution A-B, phosphomolybdic-formic-acetic formula, Bouin's and phosphotungstic solutions.

Sections, studied carefully, were not very convincing so far as localization of dye was concerned and although the same experiment was repeated many times it was finally concluded that this was not the correct approach to the problem. Attempts to cut frozen sections of unfixed blocks of tissue containing some neutral red were also not successful. Rabbits were substituted for dogs but their stomachs stained much less satisfactorily because, for one reason, it was difficult to keep them empty (sawdust often present). Occasionally a well-

stained stomach was obtained but except for some blocks which had been placed in zinc acetate (15 per cent) in Ringer's solution, frozen, cut fresh and examined immediately, very little of definite value was learned. Even these frozen sections showed only that the stain was more concentrated in muscles and surface cells, nowhere was it dense and only here and there were a few granules seen and for the most part the stain was diffuse. Counterstaining (hemotoxylin so as to get contrast with neutral red pigment) was tried but nothing of significance was learned. Cedar oil was used also.

It was then decided to study the problem with the aid of Pavlov pouches and a series of these were made in dogs. Among the first dyes tested were neutral red and Congo red. Both came through the pouch mucosa. Congo red turns blue when placed in the pouch. Trypan blue and light green did not come through. Twenty cubic centimeters of 1 per cent methylene blue, injected intravenously, appeared in the pouch in ten minutes and continued to be eliminated for one hour. Alkaline dyes on the whole seem less toxic. It is interesting that light green and methyl blue color the mucous membranes of the mouth, tongue, eyes, etc., but do not come through the pouch. Therefore the fact that methylene blue does come through cannot be explained on the basis of its generalized permeation of tissues. New methylene blue was eliminated but did not stain tissues as methylene blue did. The reactions of other dyes have already been summarized in a previous communication.

There was some difficulty in preventing gastric digestion around the Pavlov pouches. The substitution of gastrostomies was not satisfactory because the duodenal regurgitation interfered with the accuracy of mucosal elimination of dye. The feeding problem was also not as simple as with Pavlov pouches. Toward the end of the course on operative surgery when dogs were sacrificed we had the opportunity to inject dogs and observe the open stomach directly. In other words, these were not pouch observations.

It is interesting that basic fuchsin colored mucous membranes and skin but did not definitely come through the stomach. The dye was very toxic and produced shock in the animals. Upon recovery phenolsulphindophenol was injected, it was not toxic nor did it come through, but when it was repeated after being alkalinized with NaHCO_3 basic fuchsin was observed to be eliminated even though only the alkalinized phenol sulphindophenol was injected. As already noted these animals receiving basic fuchsin seemed to have their secreting mechanisms paralyzed temporarily by that dye for other dyes. In these animals there was temporary cessation of digestion of the abdominal wall due to the absence of gastric secretion. These secretions gradually returned. It seemed that the basic fuchsin had accumulated and come through later. As the secretion of digestive juices returned, acidity increased and Congo red paper was again changed to blue. Histamine was capable of producing a moderate acid flow.

All the stomachs obtained were studied after sectioning but none were entirely satisfactory except for those fixed in Susa which held the color. These sections definitely showed the neutral red. It was apparent that the pylorus and fundus were distinctly demarcated by the neutral red, the fundus stains very well, the pylorus much less so (later we concluded that this was due to

regurgitation of stain from the duodenum for when a ligature was placed at the pyloro-duodenal juncture it was not seen); there was no staining of the cardia of the stomach. The small intestine stains brilliantly, especially the duodenum; the pancreas stains well and very much less so the large bowel. Urinary system organs and liver carry the dyes abundantly.

Following a routine study of smaller laboratory animals, we decided upon the white rat as an excellent animal for this work, since the observation was made that the fundus was definitely demarcated after the injection of neutral red. The stomach, too, was small enough for fixation in its entirety. The question of fixatives again arose, but since Pfuhl⁷ had reported such excellent success with the "Susa" mixture of Heidenhain in retaining trypan blue, we decided to attempt its application to neutral red. Accordingly the whole stomachs of rats (starved for twenty-four hours) were fixed after the injection of 1 c.c. intravenously of 1 per cent aqueous neutral red in the "Susa" mixture (corrosive sublimate 4.5 gm.; sodium chloride 0.5 gm.; distilled water 80 c.c. to which was added immediately before use—trichloroacetic acid 2.0 gm., acetic acid 4 c.c., and formol 20 c.c.). They were then transferred from the fixative directly to 90 per cent alcohol and during the course of the same day through absolute alcohol. The stomachs were then carefully cut through longitudinally and passed into carbon disulphide, and then into a mixture of equal parts of carbon disulphide and soft paraffin. After eight to twelve hours in soft paraffin the halves were embedded in hard paraffin. Complete serial sections were made at 5 to 7 microns, alternate sections being stained with phloxine-methylene blue as a control on localization of the parietal cells. The experimental sections of neutral red were carried through the alcohols, cleared, and mounted in neutral balsam.

Examination of these sections showed that it was possible to retain the neutral red dye, not as pure red but as yellowish red, which could be readily detected in the canaliculi of the parietal cells. The localization of the neutral red granules corresponds exactly with the anatomic distribution of parietal cells in the phloxine-methylene blue preparations.

White rats were also used to study the selective elimination of neutral red by severing the stomach at the duodenum and at the cardio-esophageal juncture. The observation was made that the fundus stained most highly. The stomach was also severed at the fundic-pyloric juncture and at the cardioesophageal juncture. In these instances the duodenal stain regurgitated into the pylorus making it appear as if the dye were eliminated through it. Efforts were made to interfere with the blood supply to varying degrees; the more complete the interference, the less the amount of dye eliminated. The localization of the neutral red granules corresponded exactly with the distribution of parietal cells. If the cardia or pylorus seemed to contain stain in the gross, microscopic examination disclosed a few parietal or oxyntic cells to account for it. Hirabayashi's⁸ experiment, in which 0.5 per cent silver nitrate was found to prevent dye elimination and at the same time allow acid secretion, could not be verified either in the rat or dog. On the whole, the white rat proved to be a far more satisfactory experimental animal than either the dog or rabbit, although the

Pavlov pouch work in the dog could hardly have been done in the rat. On the other hand, the white rat's stomach is so small and so well demarcated anatomically (in the gross) that it allows very satisfactory observations of dye eliminations to be made. It also is more desirable than the dog's stomach for sectioning and microscopic study because the whole stomach can be sectioned in one block. This allows for an absolute elimination of parietal cells under the microscope. Such work has been done in the human being by Radasch^{9, 10} and Berger.¹¹

SUMMARY

By the use of "Susa" mixture of Heidenhain, neutral red granules were found to be selectively eliminated by the parietal cells of the white rat's and dog's stomachs. The importance of this observation becomes apparent when one considers that neutral red may be used as a test for true achylia and that its mechanism of elimination may aid in elucidating not only the pathologic changes in true achylia but in pernicious anemia as well.

REFERENCES

1. Morrison, S., Reeves, D. L., and Gardner, R. E.: The Elimination of Various Dyes From the Pavlov Pouch of Dogs (To be published in *Am. J. Dig. Dis. & Nutrition*).
2. Dawson, A. B., and Ivy, A. C.: Contributions to the Physiology of Gastric Secretion. VII. The Elimination of Dyes by the Gastric Mucosa, *Am. J. Physiol.* 73: 304, 1925.
3. Kobayashi, K.: Experimental Studies on Absorption, Secretion and Excretion. On the Secretion and the Absorption of Dyestuffs by the Stomach. I. On the Secretion of Dyestuffs Into the Stomach, *Acta scholae med. univ. imp. Kioto.* 8: 465, 1926.
4. Henning, N.: Die Ausscheidung von Farbstoffen durch die Magenschleimhaut, *Arch. f. exper. Path. u. Pharmacol.* 165: 197, 1932.
5. Forkner, C. E.: A Method for Supravital Staining of Animals With Neutral Red and Its Preservation in Paraffin Sections, *J. Exper. Med.* 52: 379, 1930.
The Origin of Monocytes in Certain Lymph Nodes and Their Genetic Relation to Other Connective Tissue Cells, *J. Exper. Med.* 52: 385, 1930.
6. Dodds, E. C., Noble, R. L., and Smith, D. R.: Gastric Lesion Produced by Extract of Pituitary Gland, *Lancet* 2: 918, 1934.
7. Pfuhl, W.: Untersuchungen über die Fixierung der vitalen Trypanblauspeicherung, *Ztschr. f. Zellforsch.* 13: 783, 1931.
8. Hirabayashi, N.: Experimentelle Untersuchungen zur Chromo diagnostik der Sekretionsstörungen des Magens, *Arch. f. Verdauungskr.* 33: 71, 1924.
9. Radasch, H. E.: The Distribution of the Acid Cells of the Stomach, *Internat. J. Gastroenterol.* 1: 24, 1921.
10. Radasch, H. E.: The Distribution of Acid Cells Along the Dorsal Curvature of the Stomach and the Possible Relation to the Occurrence of Gastric Ulcer, *Surg. Gynec. Obst.* 36: 87, 1923.
11. Berger, E. H.: The Distribution of Parietal Cells in the Stomach: A Histo topographic Study, *Am. J. Anat.* 54: 87, 1934.

THE IMPORTANCE OF THE OXYNTIC CELL IN PERNICIOUS ANEMIA*

SAMUEL MORRISON, M.D., BALTIMORE, MD.

THE story of pernicious anemia has become a familiar one to the medical profession. The gravity of the disease prior to 1926 was so well understood that a diagnosis of pernicious anemia was synonymous with death within a certain period of time except in those few cases fortunate enough to have remissions (even to this day not clearly understood) of long duration.

It is remarkable in the light of our present knowledge that so many of the early writers suspected the etiologic significance of the stomach in this disease even though during the same period every other possible etiologic agent and mechanism was at one time or another alluded to as of primary importance. It was maintained by certain investigators that all cases of pernicious anemia could not be explained on the same basis and this is also more or less realized today.

Through the maze of developments in the study of pernicious anemia many noteworthy contributions have apparently been forgotten. Among these is the excretion of neutral red as a differential test in the diagnosis of true and false achylia. Since pernicious anemia is the disease most constantly associated with true achylia, the neutral red test is valuable not only as a diagnostic measure but in the elucidation of another step in the mechanism which results in pernicious anemia. It has been repeatedly shown by other writers that neutral red is not eliminated by stomachs of patients with pernicious anemia. A review of the literature pertaining to this observation establishes it as reliable, for the dissenters are few and their work easily criticizable. Moreover our own experience would lead us to believe that neutral red is not excreted through the stomach in pernicious anemia. In some instances regurgitation from the duodenum must be considered or the position of the tube in the stomach carefully studied. When a routine procedure is adopted these disturbing factors are minimized or entirely eliminated.

We have been able to demonstrate that neutral red is eliminated selectively by the oxyntic cell of the stomach. It has also been shown that its quantitative excretion runs parallel with the hydrochloric acid secretion, i.e., it appears earlier and is excreted in larger quantities in hyperchlorhydrias than in hypochlorhydrias or normal acidities. Neutral red excretion also differentiates true from false achylia because it appears in the latter but not in the former. These results have been so definite that they have been used clinically; the correlations were made on human beings.

*From the Department of Medicine (Gastro-Enterology), University of Maryland.
Received for publication, August 15, 1935.

It would appear from the studies of Castle, Cohn and their collaborators that the intrinsic factor is entirely independent of the hydrochloric fraction of the gastric contents. Though doubtless this is true, our work leads us to believe that the intrinsic factor is a product of the oxyntic cell, not only because its secretions parallel each other in the overwhelming majority of cases of pernicious anemia but also because neutral red is eliminated by the oxyntic cell and is at the same time absent only in true achylia, which is the type of achylia without which the diagnosis of pernicious anemia is made with great hesitation. It is definitely known that neutral red and hydrochloric acid are concerned with the oxyntic cell, and now it would seem to follow that the intrinsic factor is also a product of that cell. Were this a fact one may ask why at autopsy the oxyntic cells so often appear normal. For this there is no more adequate explanation than in diabetes in which the islets of Langerhans may be just as plentiful as ever and just as normal so far as staining reactions are concerned. However, we know too little about the various granules in oxyntic cells and we are not prepared to say yet whether there is a definite group of granules associated with the intrinsic factor. We also know too little about the detection of cell sclerosis or cell senility, both factors which would influence cell secretion. We do know in the case of renal physiology that all kidney glomeruli do not function all the time, this may also apply to the oxyntic cell or the islet of Langerhans. What it is that destroys the hydrochloric acid and intrinsic secretions of the oxyntic cell so that the reaction is not reversible is still to be discovered. It has been said that stomachs in some cases of pernicious anemia have been found to re-secrete both intrinsic factor and hydrochloric acid but the work is as yet neither convincing nor acceptable. Therefore it would seem that a general destruction (not in appearance but in ability to secrete) of oxyntic cells (both gastric and aberrant) occurs, resulting in pernicious anemia. This is the usual mechanism.

According to Hurst, gastritis is a common disorder and leads to many more serious gastric disturbances among which is pernicious anemia. To us it would seem that the oxyntic cell (probably a more highly developed and complex cell) is more sensitive than either peptic or mucous cells and therefore it succumbs to the chronic irritative or inflammatory disease process present in gastritis. This is borne out clinically by the changes in acid secretion in gastric conditions, there is produced ultimately an achylia first false later true. If the gastritis is essentially an irritative, rather than an actual inflammatory, condition, the pathologic changes usually expected in gastritis may be absent and the stomach may appear normal or only slightly atrophic just as the intestinal mucosa in cases of genuine mucous colitis or as a gallbladder which to the visualization test contracts poorly or not at all seems normal on pathologic study.

CONCLUSION

The oxyntic cell possibly secretes the intrinsic factor. An understanding of the function of this cell may elucidate the mechanism of the production of pernicious anemia.

LABORATORY METHODS

THE ADAPTABILITY OF THE LIDBERG PARAFFIN EMBEDDING OVEN FOR VARIOUS TYPES OF TISSUE WORK*

CLYDE L. MATTAS, M.D., SCRANTON, PA.

THE need of new equipment for the preparation of a large number of paraffin sections at the Scranton State Hospital brought about the construction of a unit which is practical, of elastic utility, reasonably priced, and low in upkeep.

For the first section of this unit, an A.H.T. Co. No. 1785 Lidberg embedding oven, large model (Fig. 1), was selected. This oven has inside dimensions of $15\frac{1}{2}$ inches wide by $15\frac{1}{2}$ inches deep, and 18 inches high. It is made of 5-ply laminated quartered oak, making it neat in appearance and easily kept like new. It is fitted with six perforated tin shelves permitting thorough circulation of air. There is transite and hair felt insulation with thin copper lining throughout the embedding chamber.

The heating unit consists of a unique woven wire mesh embedded in asbestos between the copper apartment and the transite wall on both sides and bottom. This gives a most complete and modern method of heating the interior of the oven and is not dependent on the circulation of air currents alone.

The copper lining lends itself to the cleanliness of the interior oven, making it very easy to remove any paraffin which might accidentally be spilled in the oven.

The temperature is controlled by an expanding capsule thermoregulator with temperature range from 50° C. to 60° C., and a sensitivity of 0.5° C. The oven has the capsule adjusted for 56° C. when received, the attainment of which temperature requires approximately one hour from room temperature. The thermoregulator is situated between the walls, separated from the embedding chamber and readily accessible from the outside by means of a removable panel in which is inserted the signal lamp. This is very important as in many embedding ovens the contents must be disarranged in order to make any repairs. The thermoregulator operates equally well on either alternating or direct current. The contact points are entirely within the interior chambers so that there is probably no danger of igniting vapors from melting paraffin.

Each oven is provided with a drain hole in the center of the bottom.

The operating sensitivity, that is the maximum variation of temperature at the location of the thermometer bulb when working within the usual range, is plus or minus 0.5° C. The operating uniformity, that is the maximum variation of temperature throughout working shelf space in relation to temperature indicated by the thermometer in the interior compartment is plus or minus 2° C.

*Received for publication, June 1, 1935.

The next requirement was a drying oven (Figs. 2 and 3) capable of handling not only the regular tissue preparations on slides 1 by 3 inches in size but also larger tissue slides which are cut from glass to suit the size of the tissue, such as sections of a whole lung, or a complete section of a whole breast or kidney. And in addition to being a drying oven, it had to fulfill all the requirements and specifications of the embedding oven. This was accomplished by taking an A.H.T. Co. No. 1784 Lidberg paraffin embedding oven, small size,

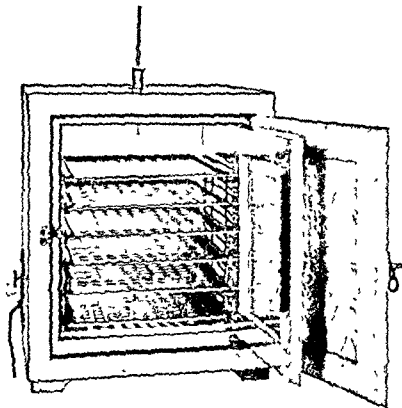


Fig 1—Lidberg paraffin oven, large model (AHT Co No 1785)

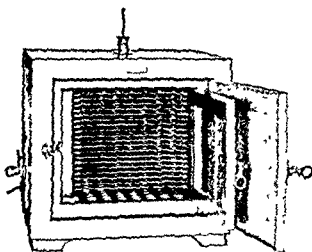


Fig 2

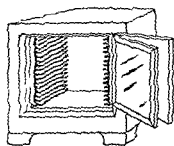


Fig 3

Fig 2—Lidberg paraffin oven, small model (AHT Co No 1784) specially fitted with 20 perforated shelves for drying.

Fig 3—Showing construction of specially perforated shelves with supports for use in Lidberg paraffin oven, small model.

with inside dimensions of 12 inches wide, 10 inches deep, and 10 inches high, and placing a new set of racks and supports in it so that 20 perforated tin shelves could be placed in the oven. This permits a very free circulation of air. The time for drying depends on the size of slide and number of slides which may be put in the incubator at any specific time.

Another essential part of the unit was a receptacle for paraffin (Fig. 4) which would keep it ready for use at a constant temperature. For this another No. 1784 Lidberg paraffin embedding oven, small size, was used. Two copper boxes, sizes $5\frac{1}{4}$ inches wide, $8\frac{1}{2}$ inches deep, and $9\frac{1}{2}$ inches high, were constructed; each was provided with a spigot. These two copper paraffin receptacles are arranged in tandem and each rests on two metal rails which prevent any leaking paraffin from holding the container to the bottom of the oven (Fig. 5). This makes it very easy to pull the receptacle out as far as is necessary for blocking tissue. The advantage is that the paraffin is always at just the correct temperature. Inasmuch as the spigot also is included in the interior of the oven, there is no difficulty with hardened paraffin blocking the spigot as is the case in many other such receptacles on the market. The advantage of having two containers instead of one is that if one receptacle becomes empty, the other is always ready to complete the blocking of the tissue without interfering with the process or making it necessary to spend considerable time in preparing the paraffin. The empty receptacle can then be filled with paraffin and can be melting while the full one is being used, thus per-

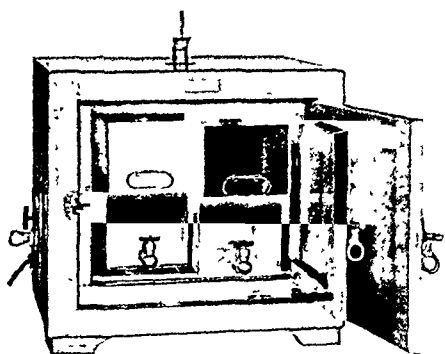


Fig. 4.

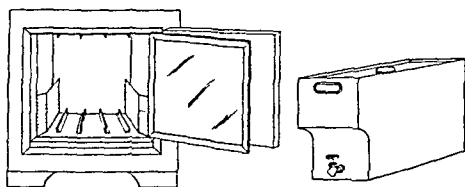


Fig. 5.

Fig. 4.—Lidberg paraffin oven, small model (A.H.T. Co. No. 1784) specially fitted with 2 copper receptacles for melted paraffin.

Fig. 5.—Showing copper receptacle for melted paraffin and alterations in the interior of Lidberg paraffin oven, small model, to accommodate same.

mitting the almost continuous use of the paraffin receptacles. Another advantage is that if a container is not available with melted paraffin in the embedding oven for a particular piece of tissue, it is very easy to select the proper sized container and fill it with melted paraffin from the receptacle.

The fact that all three sections of this unit appear alike lends to the appearance of the laboratory. Many times a laboratory will purchase units of various sizes, shapes, and colors, thus detracting from its appearance.

The shelves in the ovens are easily removable and easily replaced. Any sized receptacle can be used in the embedding oven. Any sized slide can be placed in the drying oven. The shelves on the drying oven have a guard to prevent the slides from falling and causing breakage. The paraffin receptacles not only save time but are so handy that they are a joy in the laboratory.

I am indebted to Arthur H. Thomas Co., Philadelphia, for their help and suggestions in the development of this unit in use in this laboratory. This firm is prepared to supply it on special order and intends to establish it as a catalogue and stock item if sufficient demand develops.

A METHOD FOR MEASURING THE FRAGILITY OF ERYTHROCYTES IN SALT SOLUTION*

RICHARD T. BRIDGE, M.D., AND EDWARD P. HANLEY, A.B., ALBANY, N. Y.

NUMEROUS methods for studying the fragility of erythrocytes have been described, many of which are quite accurate but rather too complex for practical application, since they involve either the necessity of colorimetric determinations or are based on changes in the H ion concentration of the solutions used.^{1, 2, 3}

The method originally described by Hamburger,⁴ and later modified by Lambeck and by Sanford,⁵ is the one in general use. It consists of adding a drop of the patient's blood to dilutions of salt solution ranging from 0.5 per cent to 0.28 per cent, and determining, after two hours, the point at which hemolysis begins and the point at which it is complete. The limitation of this method is that it occasionally gives negative results in proved cases of hemolytic jaundice, that is, it fails to detect slight degrees of hemolysis. Instances of this sort have been reported by Baty⁷ and Reynolds.⁸

With the idea of obviating this difficulty, and at the same time of preserving a simple, reliable technique, we have devised a method which seems to fulfill these requirements by further modifying the original technique of Hamburger. By applying this method to patients with hemolytic jaundice and to the study of the fragility of erythrocytes in other diseases, we have uniformly obtained clear cut results.

The steps are as follows:

1. A liter of 0.5 per cent salt solution is prepared, using dried, chemically pure NaCl and distilled H₂O.
2. 95 c.c. of the 0.5 per cent NaCl solution is diluted with 5 c.c. of distilled H₂O, making a dilution of 0.475 per cent. A buret is used to assure accuracy.
3. 0.45 per cent NaCl solution is prepared by diluting 90 c.c. of 0.5 per cent NaCl solution with 10 c.c. of distilled H₂O.

Gradually diminishing dilutions are continued in this manner, each flask containing 0.025 per cent less saline until 0.15 per cent NaCl solution is reached. These dilutions are then kept in tightly stoppered flasks, the volume of each flask having been made up to 100 c.c. as above described.

Fifteen cubic centimeters of venous blood are collected, with a dry syringe and needle, in a flask in which 8 drops of 10 per cent potassium oxalate have been evaporated to dryness. Pipette 5 c.c. of the graduated dilutions of salt solution into 14 standard calibrated 15 c.c. centrifuge tubes. To each of these tubes add 1 c.c. of the oxalated blood with a dropping pipette and invert gently several times to insure thorough mixing.

The dilutions in the tubes are:

Tube 1 contains 1 c.c. of oxalated blood and no saline. This tube is included in order to obtain a rough hematocrit reading, and serves as a base line for determining the amount of swelling of the erythrocytes before hemolysis takes place.

*From the Albany Hospital and Medical College.

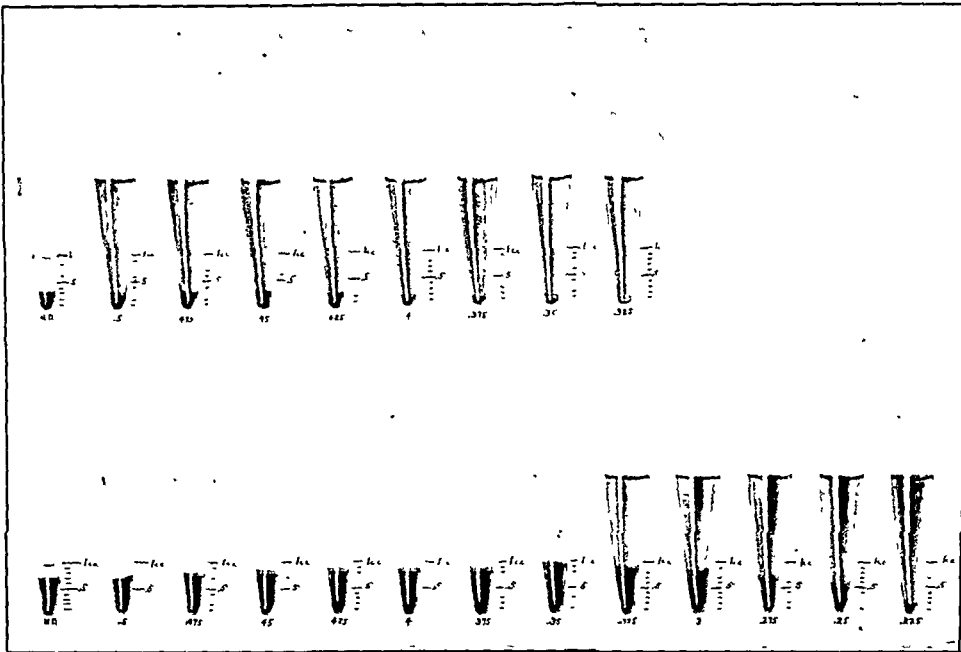


Fig. 1.—Photograph of graduated centrifuge tubes showing: (1) Rapid rate of hemolysis in severe hemolytic jaundice; (2) Delayed hemolysis in a patient with slightly increased resistance of the erythrocytes as determined by measuring the height of the column of packed cells.

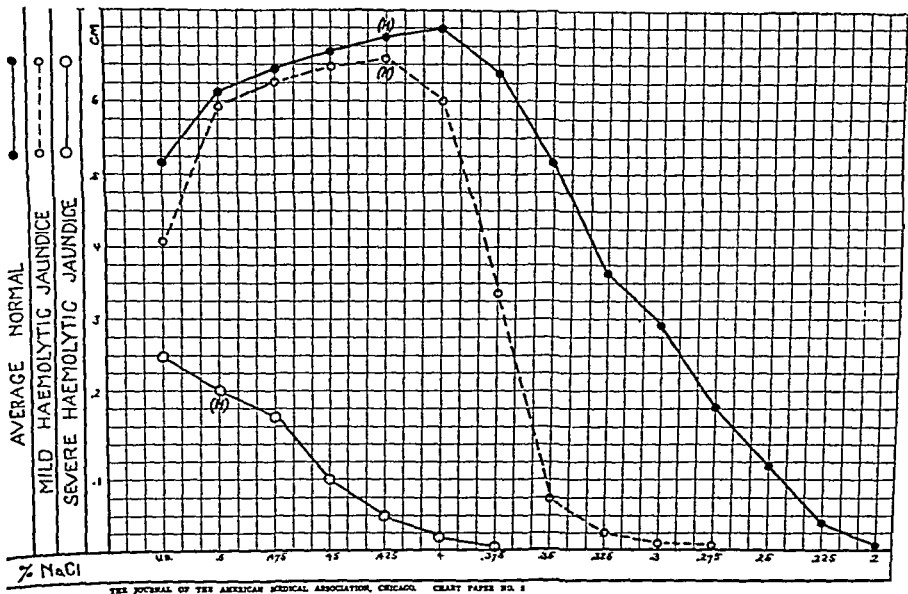


Fig. 2.—Chart showing the comparative speed of hemolysis of (a) normal blood, (b) blood of a patient with mild hemolytic jaundice, and (c) blood of a patient with severe hemolytic jaundice. The ordinate gives the height of the packed erythrocytes in centimeters, the abscissa, the dilutions of salt solution.

Tube 2 contains 5 cc of 0.5 per cent NaCl solution + 1 cc of blood

Tube 3 contains 5 cc of 0.475 per cent NaCl solution + 1 cc of blood

Tube 4 contains 5 cc of 0.45 per cent NaCl solution + 1 cc of blood

The dilutions are continued in this manner until 14 tubes have been prepared. Fewer tubes can be used in conditions where the fragility is increased, and more may be necessary in those diseases showing increased resistance of the cells.

The tubes are allowed to stand at ice box temperature for twenty-four hours, and then are centrifuged for fifteen minutes at 2,000 revolutions per minute.

In reading the results, the point at which hemolysis begins can be noted by the color of the supernatant saline, but a more accurate index of the fragility is obtained by measuring in the graduated tubes the rate of decrease in volume of the column of packed erythrocytes.

Fig. 1 shows the blood of a patient with severe hemolytic jaundice which indicates very rapid hemolysis, and the column of red cells has completely disappeared at a dilution of 0.325 per cent of NaCl solution. Compared with it is the blood of a patient with slightly increased resistance of the cells, some erythrocytes remaining in a dilution as low as 0.225 per cent NaCl solution.

The figure shows the average hemolysis curve of (1) a group of 10 normal individuals, (2) an average of 7 determinations on the blood of a patient with mild hemolytic jaundice, who, incidentally, showed no increase in fragility by the usual test⁶ for measuring the resistance of red cells, and (3) a patient with severe hemolytic jaundice.

CONCLUSIONS

This is a report of a method for measuring the resistance of erythrocytes to salt solution, which seems as simple as, and more accurate than, those now in use.

It has been possible by this test to demonstrate increased fragility of the red cells in two cases of suspected hemolytic jaundice when the usual hemolysis test has given normal readings.

A complete report on these cases and a study of the resistance of erythrocytes in other diseases are in preparation.*

REFERENCES

- 1 Saslow, G. The Effect of Washing Upon the Resistance of Erythrocytes to Hypotonic Solutions, *J. Physiol.* 74: 262, 1932.
- 2 Lepeschkin, W. W. Fragility of Red Corpuscles and Its Determination in Clinical Work, *J. LAB. & CLIN. MED.* 17: 1250, 1932.
- 3 Kunstler, W. E. Beitrag zur Methodik der Osmotischen Resistenzbestimmung von Erythrocyten *Ztschr. f. die Gesamte Exper. Med.* 93: 706, 1935.
- 4 *Arch. der Physiol.*, von Du Bois Reymond 1880, p. 476.
- 5 *Grundriss der klin. Pathologie des Blutes*, Jena: Fischer, 1896.
- 6 Todd and Sanford. *Clinical Diagnosis by Laboratory Methods*, ed. 7, p. 369.
- 7 Baty, J. M. A Case of Congenital Hemolytic Jaundice With an Unusually High Percentage of Reticulocytes, *Am. J. M. Sc.* 179: 546, 1930.
- 8 Reynolds, G. P. A Case of Acquired Hemolytic Jaundice With Unusual Features and Improved by Splenectomy, *Am. J. M. Sc.* 179: 549, 1930.
- 9 Daland, G. A., and Worthley, K. The Resistance of Red Blood Cells to Hemolysis in Hypotonic Solutions of Sodium Chloride, *J. LAB. & CLIN. MED.* 20: 1122, 1935.

*After this article went to press a paper by Daland and Worthley was published covering this subject in greater detail.

DIAGNOSIS OF CARCINOMA OF THE STOMACH FROM A FRAGMENT OF THE TUMOR OBTAINED DURING ROUTINE GASTRIC ANALYSIS*

LOUIS G. JEKEL, M.D., St. Louis, Mo.

MANY authors have cautioned against failure to examine particles of solid tissue that are aspirated from the stomach during the process of gastric lavage. Ackermann and Gompertz¹ made such a statement in 1905, and they also stressed the importance of microscopic examination of the material. Aronson,² in 1915, reporting the presence of fragments of gastric mucosa in the aspirated stomach content thirty-eight times in 400 routine gastric analyses, stated that microscopic examination proved that the tissue was obtained from the pyloric end of the stomach, and that "in at least three instances it was entirely normal." Microscopic examination did not disclose carcinoma in any one of the thirty-eight cases. Stout³ pointed out that occasionally the vomitus or the material obtained by gastric lavage may contain a shred of cancer tissue; this occurred in one of his cases, but apparently he did not report it in any current journal. Chace,⁴ in 1907, reported the diagnosis of carcinoma of the stomach from a fragment of the tumor obtained by means of the stomach tube. This is the only report of such a case that I have been able to find in any American journal of medicine or surgery since 1900. The absence of reports other than Chace's may indicate that the diagnosis of gastric carcinoma is rarely made by aspirating cancer tissue through a tube, or it may merely indicate that those physicians who have made the diagnosis in this manner have not considered the event interesting enough to report. More than this, the possibly significant inference may be drawn that the importance of the procedure may not be appreciated as fully or as widely as it should be.

The following case history is reported in the hope that physicians may recognize, more generally, the importance of carrying out all the procedures which might help them make accurate diagnoses. This patient presented a confusing clinical picture of possible carcinoma of the stomach without a palpable mass. Radiographic studies failed at first to demonstrate conclusively the presence of the tumor, and definite diagnosis was finally established by the incidental aspiration of a particle of tissue through a stomach tube during routine gastric analysis. Microscopic examination of the small bit of tissue disclosed adenocarcinoma.

REPORT OF CASE

S. L., a fifty-six-year-old, white, married coal miner, entered the hospital complaining of abdominal distention of six months' duration, loss of thirty-five pounds in weight, increasing weakness, and loss of appetite.

*From the Medical Department of The Barnard Free Skin and Cancer Hospital.
Received for publication, June 15, 1935.

Family history and past history were irrelevant.

Present Illness—About three years ago the patient began to have epigastric discomfort following meals. This discomfort later occurred more frequently and slowly changed into a definite cramplike pain which came on immediately after meals. There was some nausea associated with the pain and at times some retching but no vomiting. The patient consulted a physician who treated him symptomatically with some improvement for a short time. Six or eight months before admission his symptoms became definitely worse, and he placed himself under the care of another physician who empirically prescribed powders and two hour feedings of milk and cream. He felt somewhat better for a short time, and gained a few pounds, but soon he again became worse.

He was constipated but had never noticed bloody or tarry stools.

Physical Examination—Examination revealed a pale, haggard looking white male whose emaciation, profound weakness, anxious expression and thin weak voice gave him an apparent age of at least seventy years. He had no pain. The essential physical findings were



Fig 1



Fig 2

a scaphoid abdomen, marked epigastric tenderness, small areas of brown pigmentation on the skin and mucous membranes of the mouth, and a blood pressure of 90/60. No masses were felt in the abdomen. Examination of the head, heart, lungs, rectum and extremities revealed no abnormal findings.

Laboratory Examination—Aside from a mild secondary anemia and a positive test (guaiac) for occult blood in the stools, the laboratory findings other than the report on the gastric analysis were negative. The work up included examination of the urine, blood, sputum, and stool. Blood Wassermann and blood chemistry.

Gastro Analysis—A small nasal tube was passed without difficulty. Hypomotility and stasis were evidenced by a residual content of 200 cc. There was much mucus and blood, both bright and dark red. No free HCl was present either before or thirty minutes after the test meal. A stained smear of the sediment showed Bors Oppler bacilli. Several whitish opaque masses, each about half the size of a small pea, were separated from the remaining material because they appeared to be tissue. Microscopic examination of sections of these masses proved them to be particles of cancer tissue, and the pathologic diagnosis was adenocarcinoma.

X-ray Studies.—A gastric fill-up at the time of the patient's first visit to the clinic seemed to show a filling defect. The following morning, however, another barium meal failed to show such a defect. A third examination, after the administration of atropine, likewise failed to prove the presence of a lesion. The same results were obtained a fourth time. Serial study of these films, however, revealed an indefinite but *constant* irregularity in the region of the cardia, and led the radiologist to report "suspicious of malignancy or ulceration." X-ray of the chest was negative. All films were read by Dr. Edwin C. Ernst.

Course of Illness.—Because the patient was obviously undernourished and dehydrated, operation was postponed. The patient was placed on a high caloric, high vitamin diet, with large amounts of fluids, and in the course of about two weeks he gained ten pounds in weight and felt much better.

Exploratory laparotomy revealed generalized abdominal carcinomatosis with metastatic nodules studding the peritoneum and liver, and with a considerable amount of turbid fluid in the peritoneal cavity. Examination of the stomach revealed carcinoma in the lesser curvature extending from the cardia to the pylorus, spreading over and infiltrating both the anterior and posterior walls. The picture was practically that of a leather bottle stomach. The condition was clearly an inoperable one.

DISCUSSION

Several interesting and instructive points are brought out by this case. In the first place, although x-ray studies are an integral part of a thorough gastrointestinal work-up, they sometimes, even in the hands of an expert, do not throw as much light on the problem as is desired. It is evident, then, how difficult it might be in certain cases for an inexperienced observer to evaluate properly the x-ray findings. Also, it is clear that x-ray studies should constitute only their fair share of the work-up and should be supplemented by other procedures as indicated.

Second, gastric analysis may clear up the diagnosis in certain obscure cases. It is a simple and inexpensive procedure which can be done in the office. In spite of these facts, the value of this simple procedure is too frequently underestimated, especially by the busy general practitioner.

Finally, since normal gastric mucosa can be obtained through the stomach tube,² it is obvious that tissue from a very early cancer may likewise be so obtained. Unfortunately, in the case reported here, the diagnosis was made much too late to help the patient. Nevertheless, the possibility of an *early* diagnosis in this manner must be admitted.

SUMMARY

A case of carcinoma of the stomach was diagnosed by obtaining a fragment of the tumor through a small nasal tube. This is apparently the first time that such an occurrence has been reported in a current American journal since 1907.

REFERENCES

1. Ackermann, Wm., and Gompertz, L. M.: Microscopical Examination of the Fasting Stomach Contents and Its Diagnostic Value, *Med. Rec. N. Y.* 67: 537, 1905.
2. Aronson, E. A.: Mucous Membrane in the Fasting Stomach Content, *N. Y. Med. J.* 101: 1219, 1915.
3. Stout, Arthur Purdy: *Human Cancer*, Philadelphia, 1932, Lea & Febiger, p. 118.
4. Chace, A. F.: Diagnosis of Carcinoma of Stomach From a Fragment of the Tumor Obtained by Means of the Stomach Tube, *The Post-Graduate, N. Y.* 22: 907, 1907.

A METHOD OF STAINING PROTOZOA IN BULK*

WILLIAM S. STONE, M.D., WASHINGTON, D. C.

IN THE staining of mucous surface protozoa of man there are almost as many variations in staining technic as there are workers in the field. The chief difficulties encountered in all of the better techniques of staining are (1) keeping the organisms on the slide or cover slip during fixation and staining, (2) keeping the organisms free from distortion and clear from the debris so that their internal structures are not obscured, (3) carrying out proper differentiation of the internal structures of the organisms at the time of destaining, (4) having a sufficient number of well stained organisms on the slide after staining so that a diagnosis will not have to be made on a few more or less atypical organisms.

The following staining method not only satisfies the above requirements, but also affords a method of concentration, thereby making it possible to secure satisfactory stains from specimens containing very few organisms, that could not be stained by other methods. The method of staining differs from other standard hematoxylin staining methods only in that the organisms are not fixed and stained on slides or cover slips, but are fixed and stained in bulk. The organisms are concentrated and then carried through the steps of fixation and staining in 50 cc centrifuge tubes. Very few of the organisms present in the original specimen are lost during fixation, staining and mounting. The organisms are natural and lifelike and are not distorted by the reagents or manipulations used.

STAINING METHOD

1 *Precautions*—1 There should be no delay in concentration, examination and fixation of trophozoites after they have been secured from the patient. Needless to say, degenerated organisms will not take a satisfactory stain. It is not necessary to be so cautious in the immediate fixation of cysts, but as a rule the quality of protozoan staining possible to secure, is inversely proportionate to the elapsed time after securing the organisms from the host or culture, and their subsequent fixation.

2 Destaining must be checked by frequent microscopic observations of the organisms being differentiated. Care should be exercised to carry the destaining to the point that there is sharp differential detail between the nuclear structures and the cytoplasm. The common tendency with this technic is not to carry the destaining far enough. The organisms should be checked for structural detail after the acid destain has been neutralized and if there has been insufficient destaining they should be carried back into acid alcohol and further destained until the desired degree of differentiation has been secured.

3 The stained material must be properly dehydrated before clearing in xylol.

4 Discard the supernatant fluid decanted off after centrifuging, in each step of the procedure, or if the alcohols and stains are to be used over again, filter through a Berkefeld N filter as there is danger of carrying organisms in used alcohols and stains to the next case subsequently stained.

*From the Division of Medical Zoology, Department of Laboratories, Army Medical School.
Received for publication June 17, 1935.

5. The material being stained may be left for twenty-four to forty-eight hours' additional time in any step of the staining procedure with the exception of the fixing solution and the acid alcohol destaining reagent. This allows the staining procedure to be carried out without interfering with the routine work in a laboratory.

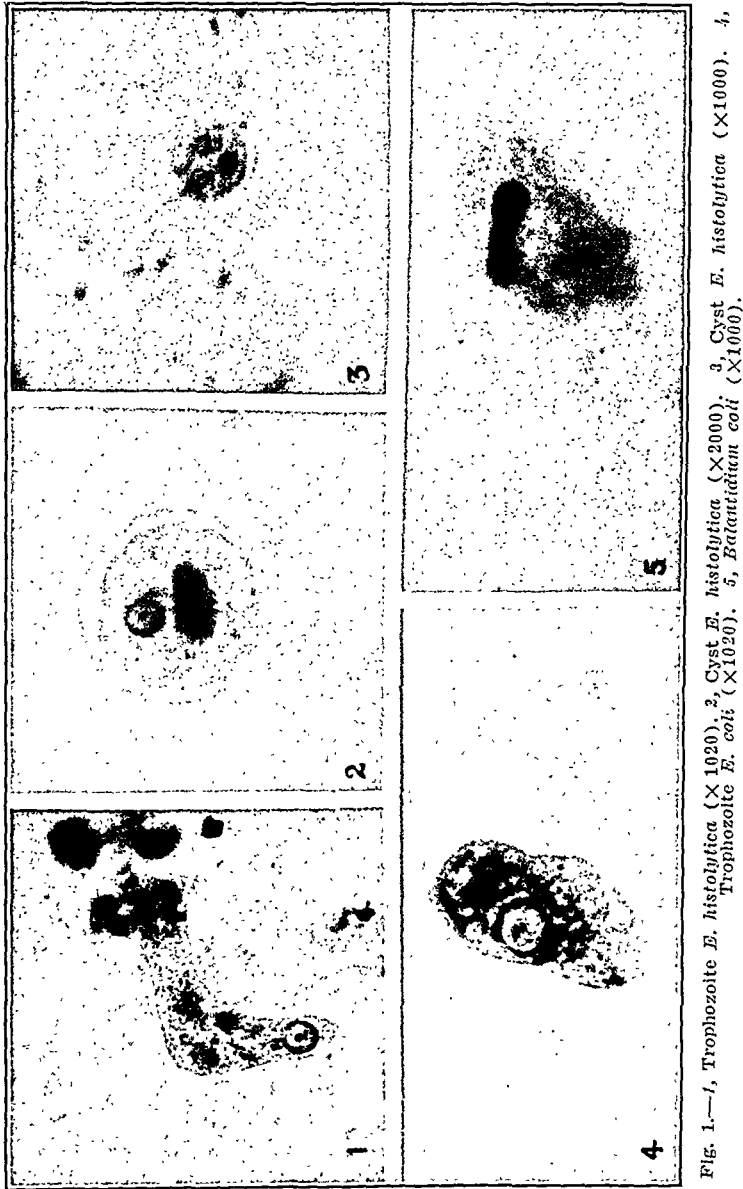


Fig. 1.—1, Trophozoite *E. histolytica* (X1020). 2, Cyst *E. histolytica* (X2000). 3, Cyst *E. histolytica* (X1000). 4, Trophozoite *E. coli* (X1020). 5, *Entamoeba coli* (X1000).

II. *Concentration and Fixation*.—1. Fecal specimens. Thoroughly emulsify 20 c.c. of feces in 200 c.c. of warm (37° C.) physiologic saline in a settling flask or tall narrow cylinder, allow to stand for five minutes and then decant the supernatant fluid into two 50 c.c. centrifuge tubes. Centrifuge the material at 1,850 r.p.m. for five minutes, then decant off the supernatant fluid, save the precipitated residue of one tube for fresh examination and to the tube containing the other precipitate add 25 c.c. of fresh Schaudinn's fixing solution.

Schaudinn's Fixing Solution

Saturated solution mercuric chloride in aqueous 0.5% sodium chloride solution	2 parts
---	---------

96% ethyl alcohol	1 part
-------------------	--------

Glacial acetic acid q s ad	1%
----------------------------	----

The alcohol and glacial acetic acid should be added to the mercuric chloride solution just before use

Thoroughly mix the precipitate and the fixative and allow the mixture to stand for at least one hour, preferably twenty-four hours

2 Cultures and other liquid specimens Pipette the fluid containing the organisms directly into a 50 cc centrifuge tube and then centrifuge and fix the material as indicated above

III *Staining*—(Between each step in the subsequent procedure the material is centrifuged at 1850 rpm for five minutes, then the supernatant fluid is decanted off and the next solution added to the precipitated residue which is then thoroughly emulsified by rotation or stirring)

1 Wash the fixed material two times with distilled water

2 Wash ten minutes with 70 per cent ethyl alcohol (containing enough Gram's iodine to give it a light brown color)

3 Wash ten minutes with 70 per cent ethyl alcohol

4 Stain by adding Harris's or Delafield's hematoxylin for from one to twenty-four hours

Harris's Hematoxylin

Hematoxylin	1 gm
-------------	------

Alcohol (ethyl 95 per cent)	10 cc
-----------------------------	-------

(Dissolve the hematoxylin in the alcohol)

Alum (ammonium or potassium)	20 gm
------------------------------	-------

Distilled water	200 cc
-----------------	--------

Dissolve the alum in the water by the aid of heat, and add the hematoxylin solution. Bring the mixture to a boil as rapidly as possible and then add 0.5 gm of mercuric oxide. The solution at once assumes a dark purple color. As soon as this occurs, remove the vessel containing the solution from the flame, and cool by plunging at once into a basin of cool water. As soon as cool the solution is ready for staining. This solution keeps for years in a well stoppered bottle (Harris)

The addition of 4 per cent of glacial acetic acid increases the precision of the nuclear staining

5 Wash with tap water

6 Destain by adding about 20 cc of acid alcohol (1 per cent HCl in 70 per cent ethyl alcohol) to the stained precipitate in the centrifuge tube. Mix the precipitate and the destaining solution and occasionally stir the mixture with a wooden applicator stick. From time to time check the progress of destaining by taking one drop of the mixture placing it on a slide applying a coverslip and then observing the progress of nuclear differentiation of the organisms under the high dry power of the microscope. The organisms will be fairly easy to find in the average case and destaining should be allowed to go on until the cytoplasm is practically colorless and the nucleus stands out sharp and clear. As soon as the required definition has been obtained add sufficient ammonia water (5 drops NH_4OH in 50 cc of distilled water) to neutralize the acid alcohol and turn the solution bright blue

7. Wash with tap water.
 8. Dehydrate with:
 - 70% ethyl alcohol 10 minutes.
 - 95% ethyl alcohol 10 minutes.
 - 95% ethyl alcohol 10 minutes.
 - absolute ethyl alcohol 10 minutes.
 - absolute ethyl alcohol 10 minutes.
 9. Clear in xylol.
 10. Thoroughly emulsify the stained cleared mixture in a minimum of xylol, breaking up all small clumps, then add sufficient thick Canada balsam to make a syrupy mixture. Then without further centrifugation,
 11. Place one drop of the balsam containing a suspension of the stained organisms on a clean slide, apply a coverslip and allow to dry.
- Permanent classroom sets and multiple permanent mounts for file and consultation can be easily prepared by making more than one preparation in the final step. Stained material suspended in balsam can be kept in bottles and mounted on slides whenever desired.

RAPID PRESUMPTIVE DIAGNOSIS OF LYMPHOGRANULOMA INGUINALE*

A SPECIFIC INTRADERMAL TEST WITH ANTILYMPHOGRANULOMA INGUINALE GOAT SERUM

JOSEPH T. TAMURA, PH.D., CINCINNATI, OHIO

SINCE December, 1933, I have been attempting to produce antilymphogranuloma inguinale serum in goats with my cultured virus of lymphogranuloma inguinale.¹

I have reported the evidence that there are in the serums of such treated animals substances which have neutralizing properties on the Frei antigen when this is used for skin tests.

During the past year I have been testing routinely all the lymphogranuloma inguinale patients, suspected cases, and normal individuals with intradermal inoculations of the antiserum specific for lymphogranuloma inguinale. Inoculations of normal goat serum and of antisera specific for other infections are made as controls. Each test is made with 0.04 c.c. of serum.

Specific skin reactions, not dependent upon serum sensitization, consist in the production of a spreading area of erythema with a slightly elevated area of central edema. The reactions generally appear within five minutes and the maximal reactions are usually reached in about twenty minutes. The erythematous areas are usually from 3 to 5 cm. in diameter. The reaction begins to disappear within twenty-five to thirty minutes after the inoculation of the serum. Intense itching and urticaria, which indicate sensitization to serum proteins, are entirely

*From the Department of Bacteriology and Hygiene, College of Medicine, University of Cincinnati, and the Cincinnati General Hospital.
Received for publication, July 2, 1935.

TABLE I
SERUMS USED FOR INTRADEPMAL INJECTIONS

PATIENT	DATE OF TEST	ANTI L I GOAT 1 10	ANTI L I GOAT 1 10 *F E A	ANTI TBC GOAT 1 10	ANTI TULARE MIA GOAT 1 10	ANTI ABOFTUS GOAT 1 10	NORMAL GOAT 1 10	FPEI ANTI GEN	CUL TUPE ANTI GEN L I VIPUS
J W	5/11/34	+		-		-		+	+
S B	5/ 3/31	+		-		-		+	+
H S	5/ 8/34	+		-		-		+	+
	2/ 4/35	+	-		-		-	+	+
R M	5/ 3/34	+		-		-		+	+
C F	5/ 4/34	+		-		-		+	+
H K	4/30/34	+		-		-		+	+
G S	5/ 4/34	+		-		-		+	+
R S	5/19/34	+		sl +		-		+	+
L A	12/ 1/34	+	-	-		-		+	+
H G	12/ 5/34	+	-	-		-		+	+
B H	12/15/34	+		-		-		+	+
L T	12/ 6/34	+		sl +		-		+	+
H W	11/26/34	+		-		-		+	+
G K	2/ 9/35	+	-	-		-		+	+
W C	1/ 5/35	+		-		-		+	+
J T	2/18/35	+	-	sl +		-		+	+
J M	1/ 3/35	+	-	-		-		+	+
C L	2/ 9/35	+	-	-		-		+	+
L G	12/26/34	+		-		-		+	+
C R	4/23/34	+		-		-		+	+
T D	4/11/34	+		-		-		+	+
B R	11/ 3/34	+		-		-		+	+
W B	2/22/35	+	-	-		-		+	+
E C	4/24/35	+	-	-		-		+	+
C B	4/ 3/35	sl +	-	-		-		+	+
F R	4/24/35	+	-	v sl +		-		+	+
C W	3/25/35	+	-	-		-		+	+
J H	1/26/35	+	-	-		-		+	+
E M	4/ 2/35	+	-	-		-		+	+
P J	5/ 9/35	+	-	-		-		+	+

Suspected Cases and Normal Individuals

W L	5/17/34	-		-		-		-	-
A N	11/22/34	-		-		-		-	-
P W	11/30/34	-		-		-		-	-
H F	2/ 7/35	-	-			-		-	-
G J	1/31/35	-	-	-		-		-	-
E J	3/ 1/35	-	-	-		-		-	-
Mc	4/ 2/35	-	-	-		-		-	-
Mit	4/ 2/35	-	-	-		-		-	-
Normal	1/30/35	-	-	-		-		-	-
Normal	1/30/35	-	-	-		-		-	-
Normal	2/20/35	-	-	-		-		-	-
Normal	2/20/35	-	-	-		v sl +		-	-
Normal	2/20/35	-	-	-		-		-	-

L I Patient Who Showed Serum Sensitivity

G M	2/ 1/35	+	+	+	+	+	+	+	+
-----	---------	---	---	---	---	---	---	---	---

*F E A indicates antiserum which has been adsorbed with fuller's earth. It has been shown by Foshay that adsorption with fuller's earth can effect a total removal of the reaction producing fraction from a number of antisera. The same is true of antilymphogranuloma inguinale goat serum. Tests with adsorbed antisera give additional controls in regard to the specificity of the reactions. It will be noted that the adsorbed antiserum gave a typical urticarial reaction in the patient who was skin sensitive to goat serum proteins.

†This normal person had been vaccinated about one year previously with a dense *B tularensis* vaccine.

absent. Tests made with normal goat serum and with goat antisera specific for other infections, e.g., antitularensis, antibrucella, antituberculosis, etc., show no erythematous-edematous reaction whatsoever.

As shown in Table I, I have tested 32 lymphogranuloma inguinale patients. Thirty-one showed specific responses, the remaining one showed serum sensitivity. All these individuals have positive Frei skin tests with Frei antigens and cultured antigens.

Foshay² has done extensive work on intradermal antiserum tests, and reported recently over 500 cases which have shown this simple erythematous-edematous reaction to the homologous specific antiserum.

Eight cases suspected for lymphogranuloma inguinale and five normal individuals were tested in like manner. No erythema-edema reaction was elicited by any of these individuals.

SUMMARY

The erythematous-edematous dermal reaction induced by the specific antilymphogranuloma inguinale serum may be taken for rapid presumptive diagnosis of lymphogranuloma inguinale.

REFERENCES

1. Tamura, J. T.: The Virus of Lymphogranuloma Inguinale, *J. LAB. & CLIN. MED.* 20: 393, 1935.
2. Foshay, L.: Intradermal Anti-Serum Tests: A Bacterial-Specific Response Not Dependent Upon Serum Sensitization But Often Confused With It, *J. Allergy* 6: 360, 1935.

A COMPARISON OF THE ZIEHL-NEELSEN AND SPENGLER TECHNIQUES OF STAINING THE TUBERCLE BACILLUS*

HERBERT WALD, M.S., AND CHARLOTTE C. VAN WINKLE, M.D.,
OAK TERRACE, MINN.

THE Ziehl-Neelsen method of staining tubercle bacilli has been generally accepted as the standard laboratory technic for the study of tubercle bacilli in sputum. Various other staining methods have been proposed and, in general, have not proved as efficient or convenient as the Ziehl-Neelsen under the test of routine use. A recent report, claiming a marked superiority for the Spengler method over the Ziehl-Neelsen on a large series of cases, was made by Koerth and Hibbard.¹ The following is an attempt to corroborate their results even though on a smaller series of smears in order to test its efficiency as a diagnostic procedure.

Specimens from positive sputum cases having relatively few bacilli per oil immersion field were selected for this study. Using the Gaffky scale, only those in Groups I and II were used with a few from Group III.

*From the Department of Anatomy, University of Minnesota, and the Glen Lake Sanatorium, Oak Terrace, Minn.

Received for publication, June 18, 1935.

All the smears were made by the two-slide method, that is, a small amount of material from the most likely portion of the sputum was placed upon a slide and another slide was rubbed over the first, spreading the material evenly over both. Great care was taken to make the smears on both slides as nearly the same thickness as possible. All slides were heat fixed and placed in cold carbolfuchsin overnight.

Ziehl Neelsen In the morning the slides were removed from the carbolfuchsin, decolorized with acid alcohol, and counterstained lightly with Löffler's alkaline methylene blue.

Spengler In the morning the slides were removed from the carbolfuchsin, placed in a picric acid alcohol* bath for five to ten seconds, washed in 60 per cent alcohol, decolorized in 15 per cent aqueous nitric acid for from ten to thirty seconds, and counterstained in picric acid alcohol for thirty seconds to one minute.

Some seventy sets of slides were prepared and examined. Ten oil immersion fields of approximately the same thickness were picked at random on each slide and all of the bacilli in these fields were counted. In the event that no bacilli were found in ten fields the slide was examined for ten minutes before it was discarded. Only the examination of the first ten fields appears in the table. In no case were bacilli found on one of the slides and not on the other, although sometimes it was necessary to examine only ten fields in one and many more in the other. It was tested and found that the relation of the slides to each other during the process of preparation (on top or bottom) bore no relationship either to the numbers or distribution of the bacilli.

Altogether 5,065 bacilli were counted on the Spengler smears, while 4,018 appeared in the Ziehl Neelsen. The average number of bacilli per field was 8.75 for the Spengler and 6.93 for the Ziehl Neelsen. The difference between these averages was 1.8 ± 2.36 which is not statistically significant. However, 39 pairs showed a difference in favor of the Spengler and of this 39, 18 were statistically significant, while only 17 showed a difference favoring Ziehl Neelsen and of these, 2 were statistically significant. Two pairs showed no difference (Table I).

The results obtained show a slight but fairly consistent superiority for the Spengler stain. However, no Ziehl Neelsen negative slides in this series were found to be Spengler positive. The superiority seems to lie in the fact that the organisms were easier to locate on the Spengler smears. It seems that in some manner not determined, more bacilli are obtained with this method. These results are not altogether in accord with those obtained by Koerth and Hibbard as they demonstrated a three to one advantage in favor of the Spengler stain as compared with the five to four advantage obtained here. The superiority of a new staining method should be greater than this to be worth changing routine and retraining technical help to install it.

It was found that occasionally the tubercle bacilli were decolorized by the Spengler technique, especially in those slides prepared with steaming carbolfuchsin instead of cold. No apparent reason for the failure was discovered. However, with some practice, and with careful handling, fairly reliable results were obtained with the steaming method. These same difficulties, though to a lesser degree, were encountered with the cold stain method.

*Picric acid alcohol consisted of equal parts of saturated picric acid (aqueous) and 95 per cent alcohol.

TABLE I

A COMPARISON OF THE AVERAGE NUMBER OF BACILLI PER FIELD ON THE PAIRED SMEARS AND ARRANGED IN ORDER OF THE VALUE OF P.

ZIEHL-NEESEN TECHNIC MEAN PER FIELD \pm STANDARD ERROR	SPENGLER TECHNIC MEAN PER FIELD \pm STANDARD ERROR	DIFFERENCE \pm STANDARD ERROR	RELATIVE DEVIATE	P \times 100
<i>Statistically Significant Differences Favoring Spengler</i>				
7.5 \pm 0.9	15.5 \pm 1.2	7.0 \pm 1.50	4.67	0.0007
6.9 \pm 1.3	23.1 \pm 3.7	16.2 \pm 3.92	4.13	0.006
2.5 \pm 0.6	6.7 \pm 1.2	5.2 \pm 1.33	3.91	0.01
1.9 \pm 0.5	12.1 \pm 3.2	10.2 \pm 3.23	3.16	0.1
0.7 \pm 0.3	4.1 \pm 1.0	3.4 \pm 1.03	3.30	0.1
4.6 \pm 1.3	11.1 \pm 1.8	6.5 \pm 2.22	2.93	0.4
1.4 \pm 0.5	5.1 \pm 1.2	3.7 \pm 1.30	2.85	0.5
1.9 \pm 0.5	3.7 \pm 0.4	1.8 \pm 0.64	2.81	0.5
7.8 \pm 2.1	18.3 \pm 3.2	10.5 \pm 3.82	2.75	0.6
1.1 \pm 0.3	3.9 \pm 1.1	2.8 \pm 1.14	2.46	1.0
0.7 \pm 0.3	4.8 \pm 1.7	4.1 \pm 1.72	2.38	2.0
1.8 \pm 0.5	4.2 \pm 0.9	2.4 \pm 1.02	2.35	2.0
0.0 \pm 0.0	1.1 \pm 0.5	1.1 \pm 0.50	2.20	3.0
4.1 \pm 1.2	11.0 \pm 2.9	6.9 \pm 3.13	2.20	3.0
4.1 \pm 0.9	6.8 \pm 0.9	2.7 \pm 1.27	2.13	3.0
0.2 \pm 0.2	2.0 \pm 0.8	1.8 \pm 0.82	2.20	3.0
0.0 \pm 0.0	0.6 \pm 0.3	0.6 \pm 0.30	2.00	5.0
2.9 \pm 0.4	4.5 \pm 0.7	1.6 \pm 0.80	2.00	5.0
<i>Statistically Nonsignificant Differences Favoring Spengler</i>				
4.1 \pm 1.5	8.2 \pm 1.7	4.1 \pm 2.26	1.81	6.0
0.7 \pm 0.3	1.6 \pm 0.4	0.9 \pm 0.50	1.80	7.0
1.5 \pm 0.3	4.4 \pm 1.6	2.9 \pm 1.62	1.79	7.0
0.0 \pm 0.0	0.5 \pm 0.3	0.5 \pm 0.30	1.66	10.0
2.6 \pm 1.3	8.3 \pm 3.6	5.7 \pm 3.82	1.49	13.0
4.2 \pm 0.9	6.9 \pm 0.8	1.7 \pm 1.20	1.42	16.0
3.3 \pm 1.5	5.5 \pm 0.9	2.2 \pm 1.74	1.26	21.0
2.3 \pm 0.6	4.0 \pm 1.4	1.7 \pm 1.52	1.12	24.0
69.5 \pm 9.6	82.5 \pm 5.3	13.0 \pm 10.97	1.19	24.0
1.5 \pm 0.6	2.3 \pm 0.5	0.8 \pm 0.78	1.03	30.0
0.4 \pm 0.2	0.9 \pm 0.5	0.5 \pm 0.53	0.94	35.0
9.3 \pm 1.7	11.8 \pm 2.6	2.5 \pm 3.14	0.80	42.0
2.3 \pm 0.4	3.6 \pm 1.0	1.3 \pm 1.07	0.77	44.0
6.4 \pm 1.4	7.7 \pm 1.1	1.3 \pm 1.78	0.73	48.0
4.0 \pm 1.5	5.2 \pm 0.8	1.2 \pm 1.70	0.71	48.0
12.3 \pm 3.3	15.6 \pm 3.6	3.3 \pm 4.88	0.68	50.0
13.2 \pm 3.3	15.3 \pm 1.6	2.1 \pm 3.66	0.63	53.0
51.9 \pm 12.1	58.3 \pm 7.4	6.4 \pm 14.23	0.45	65.0
2.1 \pm 1.0	2.7 \pm 1.3	0.6 \pm 1.64	0.37	71.0
0.5 \pm 0.2	0.6 \pm 0.3	0.1 \pm 0.36	0.28	78.0
2.6 \pm 0.9	2.8 \pm 1.3	0.2 \pm 1.58	0.01	99.0
<i>No Difference Between Spengler and Ziehl-Neelsen</i>				
1.4 \pm 0.4	1.4 \pm 0.6	0.0 \pm 0.72	0.00	100.0
5.4 \pm 1.0	5.4 \pm 0.6	0.0 \pm 1.16	0.00	100.0
<i>Statistically Nonsignificant Differences Favoring Ziehl-Neelsen</i>				
30.9 \pm 4.4	30.7 \pm 4.8	-0.2 \pm 6.51	0.03	98.0
1.5 \pm 0.7	1.4 \pm 0.7	-0.1 \pm 0.98	0.10	92.0
1.1 \pm 0.5	1.0 \pm 0.5	-0.1 \pm 0.70	0.14	90.0
17.6 \pm 5.5	16.6 \pm 2.3	-1.0 \pm 5.96	0.16	85.0
15.6 \pm 3.5	14.8 \pm 2.9	-0.8 \pm 4.54	0.18	85.0
0.1 \pm 0.3	0.0 \pm 0.0	-0.1 \pm 0.33	0.30	76.0
2.1 \pm 0.5	1.8 \pm 0.8	-0.3 \pm 0.94	0.32	74.0
16.4 \pm 7.5	12.9 \pm 3.1	-3.5 \pm 9.11	0.38	70.0
0.5 \pm 0.5	0.2 \pm 0.1	-0.3 \pm 0.50	0.60	55.0
2.8 \pm 0.9	2.1 \pm 0.6	-0.7 \pm 1.08	0.65	51.0

TABLE I—CONT'D

ZIEHL NEELSEN TECHNIC MEAN PER FIELD \pm STANDARD ERROR	SPENGLER TECHNIC MEAN PER FIELD \pm STANDARD ERROR	DIFFERENCE \pm STANDARD ERROR	RELATIVE DEVIATE	P \times 100
<i>Statistically Nonsignificant Differences Favoring Ziehl Neelsen—Cont'd</i>				
66 \pm 17	51 \pm 13	-15 \pm 220	0.68	50.0
143 \pm 29	120 \pm 18	-23 \pm 300	0.78	41.0
05 \pm 02	02 \pm 01	-03 \pm 022	1.36	18.0
13 \pm 05	04 \pm 03	-09 \pm 058	1.55	12.0
41 \pm 14	13 \pm 06	-28 \pm 152	1.84	6.0
<i>Statistically Significant Differences Favoring Ziehl Neelsen</i>				
91 \pm 23	39 \pm 06	-52 \pm 236	2.20	3.0
257 \pm 40	110 \pm 39	-147 \pm 559	2.63	0.9

The probability integral, P, is the term used to express the chance factor on a basis of unity. Hence $P \times 100$ gives the number of chances in a hundred of the observation occurring by chance alone. If $P \times 100$ is more than 5, it is commonly assumed to be the difference due to chance, if $P \times 100$ is less than 5, the difference is assumed not to be due to chance, hence is called significant.

CONCLUSIONS

No Ziehl-Neelsen negative slides in this series were Spengler positive.

The Spengler technic for staining tubercle bacilli shows a slight advantage over the Ziehl Neelsen in both the number of bacilli found and more even distribution of bacilli.

The Spengler technic is somewhat more complicated than the Ziehl Neelsen and sometimes fails to show the bacilli.

REFERENCE

1. Koerth, C. J., and Hibbard, R. J. B. Comparative Efficiency of Three Stains for Tubercle Bacilli, *J. LAB. & CLIN. MED.* 18: 535, 1933.

FROZEN STOPCOCKS*

CHARLES E. SCOVERN, JR., CHICAGO, ILL.

PLACE the stopcock in, or surround with a freezing mixture of cracked ice. Allow to chill thoroughly. After a few minutes withdraw and apply a very gentle pressure with the bare hands. Separation should come quite easily. The best results are obtained by previously heating the hands with hot water or over a flame.

This method eliminates expensive breakage and in turn prevents innumerable cut hands and fingers while doing away with the marketed so called stopcock pullers.

Stopcocks which have been subjected to strong alkalis cannot be loosened by this method nor any other. Some success is realized by applying the above to syringes as well. However, the presence of a thread or hair will prevent results. Clotted blood alone can be removed by boiling in a solution of 25 per cent glycerin before applying the "expansion treatment."

*Received for publication July 1 1935

A COMPARISON OF MEDIA FOR PLATING *L. ACIDOPHILUS**

DAVID B. SABINE, B.S., YONKERS, N. Y.

FROM time to time, there have appeared in the literature descriptions of various kinds of media especially recommended for the plating of *L. acidophilus*. Owing to the large amount of quantitative work being done in this laboratory, it was necessary to determine which of those media consistently gave best results and whether or not one medium would give equally good results with different strains.

The media used were the yeast water agar of Hunt and Rettger;¹ the cabbage juice peptone agar recommended by Bachman and Frost;² Kulp's tomato juice medium;³ the skim-milk-digest and galactose agar of Kulp and Rettger;⁴ Torrey's liver-glucose agar;⁵ the skim-milk-digest and tomato juice agar recently recommended by Kulp and White;⁶ and whey agar prepared according to Kopeloff.⁷ All media were prepared according to the original directions. In addition to these, a beef heart infusion was prepared in the usual way and 2 per cent glucose was added. All media contained 2 per cent agar; pH of media varied from 6.5 to 7.0.

Twenty-two strains isolated from widely different sources were studied. A twenty-four-hour culture of each strain was diluted and plated on each medium

TABLE I

STRAIN	SOURCE	COLONY TYPE	MOST SUITABLE MEDIUM
A	Commercial culture	Y	Liver-glucose
AO	Commercial concentrate	Y	Liver-glucose
B-330	Adult's feces	Y	Liver-glucose
3017A	Commercial acidophilus milk	X	Liver-glucose
3017B	Commercial acidophilus milk	X	Liver-glucose
3262	Adult's feces	Y	Liver-glucose
3264	Commercial concentrate	Y	Liver-glucose
3266*	Commercial culture	X	Liver-glucose
5	Adult's feces	Y	Liver-glucose
110B	Child's feces	Y	Liver-glucose
110V	Child's feces	X	Liver-glucose
180L	Child's feces	Y	Liver-glucose
180S	Child's feces	Y	Beef heart-glucose
M	Child's feces	Y	Beef heart-glucose
3010	Commercial culture	Y	Beef heart-glucose
3268	Commercial culture	Y	Beef heart-glucose
3270	Commercial acidophilus milk	X	Liver-glucose
3269†	Commercial culture	X	Liver-glucose
3272A	Commercial culture	X	Liver-glucose
3272B	Commercial culture	Y	Liver-glucose
3267	Commercial acidophilus milk	X	Liver-glucose
SM	Commercial acidophilus milk	X	Skim-milk-digest

*Commercial culture of *L. bulgaricus*.

†Labelled acidophilus but its cultural characteristics were more closely allied to bulgaricus.

*From the Laboratories of the Arlington Chemical Company.

Received for publication, June 27, 1935.

and the plates incubated for seventy-two hours. The medium showing the highest count was chosen as the most suitable for that strain. Each individual count was the average of three plates and each test was repeated three times. In this way, experimental error was eliminated as far as possible. Table I shows the results.

Little mention has been made in recent years of Torrey's liver glucose agar for *L. acidophilus*. Although the above results indicate that no one medium can be selected as the most ideal for all types and strains, the fact that 17 out of 22 of the strains studied grew better on this medium than any other is worthy of more than passing notice.

The medium is easy to prepare, being simply an infusion of liver with 1 per cent glucose and 0.1 per cent K_2HPO_4 added. The pH usually requires no adjustment, falling in the range 6.5 to 7.0. It has a brown color against which the colonies stand out, making counting much easier than on lighter colored agars. Both the filamentous and smooth type of colony grow rapidly and to a large size in this medium. Plates may be counted accurately with the unaided eye after forty-eight hours' incubation. It is also a very satisfactory medium for direct isolation either as prepared or slightly acidified to inhibit contaminating organisms. The author has used this medium almost exclusively for the past four years. In thousands of routine examinations of pure cultures for viability counts and also in many direct isolations, it has given eminently satisfactory results.

REFERENCES

1. Hunt, G. A., and Rettger, L. F. A Comparative Study of Members of the *Lactobacillus* Genus With Special Emphasis on *Lactobacilli* of Soil and Grain, *J. Bact.* 20: 65, 1930.
2. Bachman, F. M., and Frost, W. D. Vegetable Peptone Agar for Quantitative Work With *L. Acidophilus*, *J. Bact.* 23: 39, 1932.
3. Kulp, W. L. An Agar Medium for Plating *L. Acidophilus* and *L. Bulgaricus*, *Science* 66: 512, 1933.
4. Kulp, W. L., and Rettger, L. F. Comparative Study of *L. Acidophilus* and *L. Bulgaricus*, *J. Bact.* 9: 364, 1924.
5. Torrey, J. C. New Differential Plating Methods for *B. Bradius* (Tissier) and *B. Acidophilus* (Moro), *J. Bact.* 2: 439, 1917.
6. Kulp, W. L., and White, V. A Modified Medium for Plating *L. Acidophilus*, *Science* 76: 17, 1932.
7. Kopeloff, N. *The Lactobacillus Acidophilus*, Baltimore, Williams and Wilkins Co., 1926, p. 195.

A METHOD OF THREE COLOR PHOTOGRAPHY*

ADAPTED TO PHOTOMICROGRAPHY, LANTERN SLIDES, AND PAPER PRINTS

OLAN R. HYNDMAN, M.D., IOWA CITY, IA., AND TRACY PUTNAM, M.D.,
BOSTON, MASS.

IN PHOTOGRAPHY intended for medical illustration and teaching, there often arises the need for color differentiation. Particularly is this true in photomicrography. True color rendition is not necessary. The advantage lies in a differentiation of the elements of the tissue by color, the goal that is striven for in differential staining of the section itself.

At present there are two general methods of recording color photographically.†

1. The screen plate or autochrome method: In this case a mosaic of very small filters (red, blue and green) is in close contact with a thin, fine-grained panchromatic emulsion. The screen plate may be (A) fixed to the emulsion permanently or (B) a separate plate which may be registered at will in taking and viewing the picture.

Available types under A are:

Lumièrecolor in which the mosaic screen is composed of dyed starch grains. These films give beautiful natural color transparencies but are too opaque and heat labile for satisfactory projection with the ordinary equipment.

Agfacolor in which the screen is composed of fine gelatin granules dyed red, green, and blue. This plate requires about half the exposure time and is more transparent than *Lumièrecolor*. Both of these are likely to melt when projected unless means of cooling is provided.

Dufaycolor, a promising film recently placed on the market whose screen is composed of a mosaic of dyed squares (about one million to the square inch). The exposure time is short, about four times that of supersensitive panchromatic emulsion and the transparency is quite satisfactory for ordinary projection. The film is available at the present time only in 35 mm. (Leica) size.

In the "A" type of screen plate the positive is obtained by reversing the negative.

The "B" type is represented by the Finlay method. The taking screen, a mosaic of equi-dimensional squares, is placed in contact with a panchromatic plate. The negative is developed and the positive made from this is registered with a viewing screen. These are quite transparent and heat resisting. An

*From the Neurological Unit, Boston City Hospital, and the Department of Neurology, Harvard Medical School.

Received for publication, July 1, 1935.

†We are purposely omitting the additive methods of which *Kodacolor* is an example inasmuch as these are more adaptable to motion picture projection.

advantage has been claimed in that a few viewing screens will suffice for an unlimited number of pictures and thus entail expense. We find it distinctly inconvenient, however, to have to register and bind a group of lantern slides whenever they are to be shown. It is much more desirable to have the slide finished and always ready for use. The cost would usually prohibit the permanent binding of Finlay screens to a large number of lantern slides. The mosaic is sometimes apparent to an annoying degree on projection.

In addition to those already mentioned, we must say that the chief disadvantage of all screen plate methods excluding the Finlay method lies in the extremely small latitude of exposure. It is easy to over or underexpose and little can be done to retrieve such a mistake. Particularly is this true in photomicrography where the exposure varies markedly from section to section and the exact exposure is difficult to determine. The cost of the material is considerable. Duplicates can be made but, excluding the Finlay method, each duplicate will be as expensive as the original and under the best conditions seldom as good.

A minor but definite criticism of all the above screens is the fact that the colors will fade to some extent after long exposure to strong light.

The second general method of recording color is by taking three "separation" negatives each through a primary color filter respectively, red, green, and blue. Unless one is equipped with a 'single shot' three color camera, the negatives must be taken separately, but in photomicrography this is entirely feasible. Gelatin reliefs are made from the negatives, dyed a color complementary to the taking filter and registered to produce the three color transparency.

In order to overcome some of the disadvantages given for the other methods, we have worked out a process utilizing separation negatives. We have followed the "Pmatype process" as outlined by the Eastman laboratories with some modifications, especially in that we are suggesting a new and satisfactory set of inexpensive dyes available in this country. We endorse this method as having the following advantages: greater control and latitude of exposure, elimination of correction filters, unlimited duplication, possibility of enlargement from miniature negatives, the possibility of selective color intensification, transparency equal to the black and white lantern slide, heat and light resistance, possibility of color prints on paper, and inexpensiveness.

Method.—Taking the negatives. Any camera with a good lens may be used. Concerning the camera lens much is said of the necessity of apochromatism but excellent results can be had with the use of a good achromatic lens. Needless to say a high quality microscope will give better results in any type of photomicrography. In the Department of Neurology of the Boston City Hospital, the Leica with "microirbo" microscope attachment was found to be most economical in film expense, time, and labor. Three "separation" negatives are taken of the subject through Eastman filters A, B, and C,* which are red, green, and blue, respectively. The filter may be placed anywhere between the source

*This is known as the Eastman tricolor set. The dyes to be subsequently used were selected to be used in conjunction with these filters. Throughout the monograph an order is proposed in which the three components of the picture may be taken, printed and dyed. Some routine order should be strictly adhered to.

For soft negatives use A, 2 parts, B, 1 part, and C, 2 parts. This proportion is suggested for average subjects. Develop five minutes at 65° F.

For contrast use A, 1 part, B, 2 parts, and C, 4 parts.

A gives a soft result, while B and C give contrast so that by mixing in different proportions, any desired result may be obtained.

This developer may also be used for paper, but since it contains no potassium bromide, a drop or two of a 10 per cent solution should be added to each ounce of final solution. The negatives are fixed and dried as usual.

Making the positive reliefs. The Eastman Kodak Company has placed on the market a "wash off relief positive film" especially adapted for this purpose. It consists of a thin celluloid base coated on one side with a positive emulsion sensitive only to blue violet light and of about lantern plate speed. It can be used for contact printing or enlarging. Briefly a positive is made by exposing through the celluloid side of the film. This is developed, washed and bleached

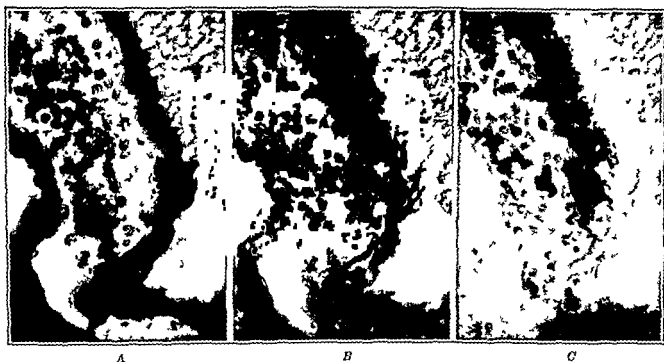


Fig 1—Prints from three separation negatives made with a Leica micro lens attachment according to the method described in the article. The illustration shows a platelet thrombus in a blood vessel from a case of multiple sclerosis. Masson connective tissue stain. Oil immersion lens. The vessel wall is blue erythrocytes are orange platelets are purplish and the surrounding tissue is brown. A is to be stained blue. B is to be stained red. C is to be stained yellow. When the three are superimposed as in a lantern slide the colors in the original preparation are closely imitated.

in a special solution which tans (renders insoluble in hot water) the gelatin wherever there is reduced or metallic silver and in proportion to the amount of silver deposit. The remainder of the gelatin is still soluble in hot water so that if the bleached positive is immersed in warm water the soluble gelatin will wash off leaving a gelatin relief which when dyed will be a positive image.

The positive film is exposed through the celluloid side so that the relief will be adherent to the celluloid base after having been washed down. If exposed in the ordinary way, the entire emulsion will wash off.

It is most important that the relief should be thin and flat. No part of the relief should extend the entire thickness of the gelatin. To put it another way, part of the entire surface of the gelatin should come away when washing down. "Flat" in reference to the relief has the same connotation as "soft" in refer-

ence to a negative and this quality is attained by the use of very soft negatives plus a special property of the wash-off film. In addition to the emulsion being sensitive to blue violet light only, the gelatin is dyed yellow. Yellow absorbs violet and hence the only light to which the emulsion is sensitive is held back and not permitted to penetrate more than a short distance. This can be accomplished to a still greater degree by filtering the printing light through a Wratten violet filter No. 35 and this is advised. Dyed reliefs tend to be contrasty, and one is often surprised at the brilliant contrast obtained in a relief prepared from what appeared to be a hopelessly flat negative.

Another principle in making the relief that must be rigidly adhered to is that the highest light in the positive must be just veiled over. That is, when the positive is developed, the lightest place in the picture must develop to a light gray so that when the film is washed down, there will be no absolutely clear spaces devoid of gelatin in the picture. This is accomplished by the proper printing exposure and developing time. It is well to keep the developing time a constant as given in the method and vary only the printing exposure.

This axiom has one exception. For scenes in nature the rule holds since in a natural scene there are no holes or voids and every spot on the picture must represent some part of the scene. However, in photomicrography there are often holes or voids in the section which should have no representation in the picture except as what they are—a blank space. If one attempts to push the printing exposure so as to veil over these places, the remainder of the relief will be too thick and its quality impaired. In this instance we should say that the highest light in the tissue should be just veiled over.

Method.—1. Expose the “A” (red filtered) negative on the celluloid side of the positive film by contact or enlargement. This may be determined by exposure meter such as the Largodrem or trial and error. All three separation negatives, however, should receive the same exposure.

*2. Develop, under a safe yellow light, eight minutes in the following developer, frequently rocking the tray:

Eastman D-16	
Water (about 120° F.—52° C.)	2,000.0 c.c.
Elon	1.2 grams
Sodium sulphite	160.0 grams
Hydroquinone	24.0 grams
Sodium carbonate	75.0 grams
Potassium bromide	3.6 grams
Citric acid	2.8 grams
Potassium metabisulphite	6.0 grams
Cold water to make	4,000.0 c.c.
Develop at 65° F.—18° C.	

3. Wash two minutes in running water.

4. Bleach one to three minutes in the following solution:

A. Ammonium bichromate	20 grams
Con. sulphuric acid	14 c.c.
Water to make	1,000 c.c.
B. Sodium chloride	48 grams
Water to make	1,000 c.c.

*The image will appear on the celluloid side of the film. It should never be more than faintly visible on the reverse side.

Use 1 part of A, 1 part of B and 6 parts of water Not more than three films should be bleached in 150 cc of solution

Water	425 0 cc
Potassium bichromate	15 grams
Potassium bromide	50 grams
Glacial acetic acid	150 cc

The solution will keep indefinitely and may be used repeatedly

The positive will not be entirely bleached and a faint image will remain when viewed through the celluloid side of the film, but it will be evident when the process is at an end

In the process as described by the Eastman Company, the positive is fixed in a nonhardening fixing bath, then bleached and washed down There is a distinct advantage in bleaching and washing down before fixing in that the unused silver halide enables one to tell more easily when the washing down is complete as well as to observe the quality of the relief

5 The film is transferred directly from the bleach to warm water at about 110° F -43° C and kept in motion The washing off process is hastened by dipping the film in and out of the water occasionally It is well to use a large vessel for this purpose The film should be washed until milky gelatin ceases to drip off Dip in cold water and examine it toward the light to see if the density and quality are satisfactory

6 Fix in ordinary hardening hypo, wash thoroughly (fifteen minutes in running water), and allow to dry

It is almost necessary to handle the edge of the film throughout its development with some sort of clamp A hemostat is ideal, though a spring clothespin is satisfactory The relief is extremely delicate and will scratch if it comes in contact with anything but the solutions

A good routine is to print the negatives in the order that they were taken A, B, and C Both negatives and positives should have some mark of identification A satisfactory method is to mark one, two, and three dots or short lines with ink in one lower corner on the emulsion side of the negative to represent the A, B, and C negatives, respectively These marks should register on the positives and serve to identify them

Dyeing the reliefs* Obviously each relief must be dyed a color complementary to the filter through which its negative was taken That is, the "A" positive should be dyed a greenish blue the "B" positive a blue red, and the "C" positive yellow The following dyes fulfill these as well as certain spectroscopic requirements and are fast to light Gelatin imbibes them rapidly and they transfer satisfactorily to gelatin coated paper The three dyed reliefs are superimposed so that the three colors overlap each other Each dye, therefore, must not only impart its color but must have spectral transmission properties

*The dyes may be obtained inexpensively from the following companies

Pedt Brilliant sulphon red 5 B

Blue Pyrazol fast blue 4 GL

Sandoz Chemical Works Inc 36 Purchase St. Boston Massachusetts

Yellow Tartrazine S E—Geigy Company Inc 88 Broad St Boston Mass

†We wish to thank the Sandoz and the Geigy Companies for their cooperation in supplying us with samples of many dyes We also wish to thank Mr William H Kunz 1103 Boylston St Boston Mass for suggestions pertaining to color photography

that will allow each point in the tricolor picture to be a facsimile in color to the original. Authorities in color perhaps rightly claim that a facsimile is impossible when using only the three primary colors. Results, however, can be such as to fall little short of perfection to the naked eye.

Aside from the concentration of the dye, two substances will influence the depth and rapidity of staining. Acetic acid causes the gelatin to imbibe the dye more deeply and quickly. It should, however, never be greater than 1 per cent in concentration. Potassium citrate will produce a lighter and more penetrating stain.

The dyes should be allowed to dissolve in tap or preferably distilled water, then filtered through fine cloth.

Method.—It is well to have two working sets of dyes: a weak set for reliefs that are on the thick side and where softer shades are desired, and a strong set for reliefs that are on the thin side or where deep staining is desired. After some experience the red and blue components may be selectively stained more deeply to place the desired emphasis and contrast on certain tissue elements.

The following two working sets of dyes are suggested:

<i>Weak set:</i>			<i>Strong set:</i>		
Blue	{ Dye	0.25%	Blue	{ Dye	0.5%
	{ Acetic acid	0.5%		{ Acetic acid	0.5%
	{ Pot. citrate	3.0%			
Red	{ Dye	1.0%	Red	{ Dye	2.0%
	{ Acetic acid	0.5%		{ Acetic acid	0.5%
	{ Pot. citrate	1.0%			
Yellow	{ Dye	1.0%	Yellow	{ Dye	2.0%
	{ Acetic acid	0.5%		{ Acetic acid	0.5%
	{ Pot. citrate	1.0%			

Use: A, 1 part; B, 2 parts; and water 64 parts.

The relief prepared from the "A" (red) filtered negative is stained blue, that from the "B" (green) filtered negative is stained red, and that from the "C" (blue) filtered negative is stained yellow. The dye should cover the relief well and be occasionally rocked. Staining will usually be complete in about five minutes. After washing the excess dye from the relief in running water, the remainder may be permanently fixed by dipping the relief in 2 per cent acetic acid. It is then washed free of acid and dried.

If there seems to be difficulty in getting the dye to penetrate into the relief, immerse the latter for a minute or two in the following solution:

A. Water	1,000.0 c.c.
Pot. permanganate	52.5 grams
B. Water	1,000.0 c.c.
Sulphuric acid (conc.)	32.0 c.c.
Use: A, 1 part; B, 2 parts; and water 64 parts.	

Then clear in a solution of sodium bisulphite, one part to fifteen, wash and stain. When the three reliefs are stained and dried they are registered and the result examined against the light.

It will be found easier to register the yellow and blue first, preferably by daylight. The blue and red will be found to differ little from artificial to day-

light but yellow appears much more intense by daylight. The two are held by a hemostat or stout paper clamp on the edges while the red relief is registered, then all three clamped together. If satisfactory, the corner opposite the clamp may be glued with a film glue made by dissolving 3 or 4 square centimeters of celluloid film in 10 or 15 cc of acetone. Apply glue between the first and second, then second and third relief corners pressing firmly for a minute between each application, making sure register is maintained.

If one of the colors appears disproportionately dark when the three are superimposed, the corresponding film may be removed and washed down a little further in hot water. Contrast may be increased by adding a small amount of borax to the water.

When the reliefs are dried, they will be found to have a slight milky opacity which will hinder projection to some extent and necessitates clearing. The opacity is common to all bleached silver tanning methods and for this reason we are describing an alternative method of preparing reliefs below for those who would prefer it.

The above reliefs are cleared by covering the matte surfaces of each with a varnish suggested by the Eastman Kodak Company. It is flowed on the matte surface of the relief and after draining off the excess, allowed to dry thoroughly (several hours) in a well ventilated room. The three cleared reliefs may then be registered and bound between lantern slide cover glasses. The varnish is prepared as follows:

Gum sandarac	25 grams
N Butyl alcohol	200 cc
Castor oil	5 cc
Oil of lavender	1 cc

The gum sandarac and butyl alcohol are warmed together until the gum is dissolved, avoiding an open flame as butyl alcohol is inflammable. Filter through a fine, lintless cloth mix the other ingredients and cool.

Alternative method of making reliefs which precludes the necessity of final varnishing

This is known as the bichromated gelatin method and depends upon the fact that when gelatin is sensitized with potassium bichromate it becomes sensitive to light so that wherever it is acted upon by light, the gelatin becomes insoluble in warm water. Since it is not practical to attempt enlarging by this method, the separation negatives must be made the size of the finished picture.

Any single coated film may be used. Some gelatins are inherently harder than others but this can usually be allowed for. It does not matter if the emulsion is light struck since we are here using the silver halide only as a pigment to be subsequently removed and not as a light sensitive salt. We are interested only in the gelatin of the emulsion. Wash off relief film is ideal, however, for this purpose since the gelatin is soft, the celluloid support is thin and clear and it already contains a yellow dye.

Single coated discarded x-ray film may prove in some places to be an inexpensive source for practice material. It has the disadvantage of having a rather heavy celluloid support which has a light bluish cast. It will prove satis-

factory for 5" × 7" or 8" × 10" reliefs to be printed on paper. Most x-ray film at the present time is double coated but the emulsion can be removed from one side with warm water to which a little sodium bicarbonate has been added (a teaspoonful to the pint). During this procedure, the reverse side must be kept dry.

The gelatin must contain a yellow dye for the same reason as outlined for the other process. Bichromated gelatin is also sensitive to violet light only.

Method.—The film is first immersed in the strong yellow dye which is used for dying reliefs (Tartrazine S.E. 2 per cent in 0.5 per cent acetic acid) for about three minutes.* Allow to drain a few seconds and immerse for three minutes in the following sensitizer:

Quick drying sensitizer:

Water	700 c.c.
Potassium bichromate	28 grams
Methyl alcohol	700 c.c.

The sensitizing procedure may be carried out in subdued white light or abundant yellow light as the bichromated gelatin is practically insensitive when wet.

The sensitized films are now hung in a dark place to dry. They should be used within a few days after preparation though they will often remain good for several weeks.

The dry film, which must now be handled in yellow light, is laid emulsion side down and with a moist cloth the celluloid side is wiped clean of debris and polished.

Exposure.—Here again the exposure is made through the celluloid side of the film. A contact print is made in daylight, arc or photoflood light. Do not expose in direct sunlight. The exposure time for any given light conditions and negative will have to be determined. However, in the bright shade of a brilliant day, using an ordinary negative, the exposure will be of the magnitude of ten to twenty seconds.

The print is washed down in warm water as described for the previous method.† The yellow dye will wash out and the silver salt in the gelatin will serve to outline the picture which should fulfill the requirements as given for the other method.

The relief is fixed in ordinary hardening hypo, washed thoroughly and allowed to dry. When dry there should be no visible image. Hence a special precaution such as notching the edge of the film should be taken in order to identify the respective reliefs.

The procedure from here on whether for transparencies or paper prints is identical with that described for the other method except for the fact that varnishing is not necessary.

*If Eastman wash-off relief positive film is used, it will not be necessary to dye it since it contains a yellow dye.

†If the gelatin used seems too hard to dissolve, add a little sodium carbonate to the warm water.

Method of making imbibition prints on paper—The reliefs are dyed as already described. Almost any gelatin coated paper will be satisfactory though some seem to be of such hard surface as to require a longer time for dye transfer than others. We recommend the autotype double support final transfer paper, smooth surface*.

The usual smooth surface printing papers can be used of which we find matte azo paper the most satisfactory. The printing papers should be fixed in plain hypo (without hardener) and washed.

Soak the gelatin coated paper ten or fifteen minutes before using. It is better to soak glossy papers in warm or even hot water.

Place the paper in contact with the blue relief under water and pull the two out so as to exclude all air bells. Place the two papers down on a moist blotter which is in turn on a flat surface. While the edge of the relief is held down firmly with the fingers of the left hand, it is squeezed to the paper with a flat squeegee or a soft towel wrapped around the first and second fingers of the right hand. Stroke only one way and firmly enough to exclude all excess water. Then squeegee in the other three directions, place a flat glass over the film, and about a quarter to a half pound weight on this. On soft gelatin paper the transfer will be made in about ten or fifteen minutes. A good typewriter board fitted with a stout clamp on one end will be found useful for holding the relief while it is being squeezed.

The relief is returned to the dye bath if more prints are to be made and the paper is immersed for one minute in 2 per cent acetic acid to fix the dye. This keeps the image sharp and prevents any of the blue dye from transferring to the second relief.

The blue relief must be transferred first. Not only will this give better color value and make subsequent registering more easy but once the gelatin coated paper is soaked in acetic acid, the blue dye will not transfer.

After washing the acetic acid from the blue print, transfer the yellow in like manner. Here, again, registering of the yellow will be found easier in day light. The blue yellow print is soaked in weak acetic acid as before, washed, and the red transfer made. The print should not be allowed to dry between transfers.

If the reliefs should become deeply stained after much use so as to make registering difficult or if they should become mottled from an uneven transfer, the dye can be removed in the permanganate bath given under the method of dyeing reliefs. Place in permanganate bath a minute, rinse and clear in the bisulphite bath, rinse and reimmerse in permanganate if necessary. After being cleared, they are ready to be redyed.

We have endorsed the above three color process and have omitted various two color processes purposely. It requires almost as much effort to make a two color preparation as a three color one. To juggle dyes in each instance that might simulate the tissue stains removes the procedure too far from standardiza-

*This may be obtained from George Murphy, Inc. 57 East 9th Street New York, N. Y. or in large rolls from The Autotype Company 74 New Oxford Street London W. C. England

tion and is not at all necessary. Once the three color procedure is established as a routine, the only real thought necessary to give it is in exposing the separation negatives.

Though the procedure as given here may appear involved, after a few trials (observing routine arrangements) it becomes relatively simple and not very time consuming. The instruction has purposely been made detailed in the hope that others will avoid some of the pitfalls into which we stumbled.

AN INEXPENSIVE LABORATORY TIMING DEVICE*

NORMAN A. DAVID, M.D., AND RALPH A. LINCOLN, CAPT., ENGR. CORPS., U. S. A.,
MORGANTOWN, W. VA.

EVERYONE visiting physiologic laboratories in this country seems to recognize that there is still need for a simple and inexpensive laboratory timing device to replace the complicated or inadequate equipment now used. Two types of timing apparatus are employed at present: the watch with attached writing lever and the central timing clock or apparatus which is connected electrically to operate a number of signal magnets. The advantages and disadvantages of these devices are discussed below for comparison with the new timing machine described in this paper.

STANDARD TIMING DEVICES†

The inexpensive Lieb-Becker type of timing watch is well suited for recording time intervals when single kymograph drums are used. This watch, however, is delicately constructed, requires frequent adjustment, and will not write unless the lever is very lightly applied against the recording paper. Supplying large student laboratories with a sufficient number of these watches is an expensive item.

An example of the central timing apparatus is the ordinary wall clock with a small wire-tipped pendulum. The pendulum is connected in circuit with dry cells and one or more signal magnets, the circuit being closed each time the wire tip of the pendulum swings through a cup of mercury placed at the bottom of the arc. Usually, with this arrangement the only time intervals obtained are those of one-half second which are difficult to count on a slowly moving drum. A much more satisfactory, as well as more variable, device is the Brodie pendulum time marking electro-magnetic apparatus. It is expensive, however. The Jaquet

*From the Department of Pharmacology, University of West Virginia School of Medicine. Received for publication, July 3, 1935.

†Obtained from A. H. Thomas and Co., Philadelphia, Pa., and other laboratory supply houses. Cost of Lieb-Becker Timing Watch \$11.00; Brodie Pendulum Electro-Magnetic Apparatus \$111.30; and the Jaquet Chronometer \$70.00.

graphic chronometer may be similarly employed to operate signal magnets when connected in circuit with dry cells, although it is inadvisable to use more than one dry cell with this sensitive watch

DESCRIPTION OF NEW TIMING DEVICE

An accurate and reliable timing device possessing several advantages not found in the apparatus described above may be inexpensively assembled as shown in Fig 1 from a few gears and a small synchronous electric motor. Patterned after the household electric clock, its operation is quite simple yet very positive and accurate. Suitable reduction gears are used to reduce the speed of the motor to one revolution per minute or to any other ratio desired. A drum wheel attached to the shaft turning one revolution per minute is provided with a certain number of teeth, pins or notches which operate the pivoted arm of a

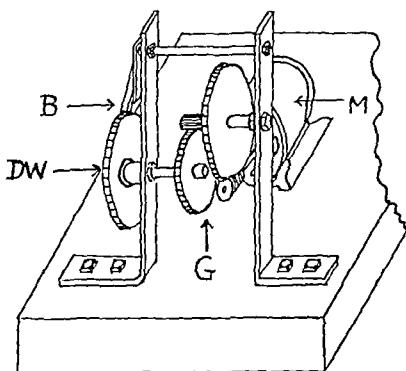


Fig 1—Diagram of assembly of motor driven timing device. *M* synchronous motor. *G* reduction gears (inclosed in oil reservoir). *DW* drum wheel with fifty nine notches. *B* pivoted breaker arm.

current "breaker." The "breaker" is placed on a frame directly over this wheel and as the wheel turns, it is raised by the teeth or dropped into notches at certain regular intervals of time. When the "breaker" is either raised or lowered, platinum contact points are brought together and an electrical circuit is closed. Included in the circuit are dry cells and a number of signal magnets with attached writing levers. Further detailed specifications for the construction of such a device are given below.

SPECIFICATIONS

A The Motor.—The use of a synchronous motor for running this device is necessary since with this type the speed is always maximal and is not altered by the load. Small motors of from one fiftieth ($\frac{1}{50}$) to one twentieth ($\frac{1}{20}$) horse power are preferable although larger ones of from one sixth ($\frac{1}{6}$) to one

fourth ($\frac{1}{4}$) horse power will do. These motors can be operated on the usual 110 volt alternating current. Since they are used commercially, they are readily obtainable, new or secondhand.

B. Speed Reducing Gears.—Appropriate gears are used to reduce the speed of the motor, which is usually 1800 R.P.M., down to 1 R.P.M. or to any other ratio desired. Compact reducers with the gear train inclosed and running in a reservoir of oil are carried in stock by certain supply houses.* These are readily attached to the motor and held in position by a metal frame. If silent operation is desired, fiber gear wheels may be substituted.

C. The Drum Wheel.—This wheel is from eight to ten inches in diameter and is attached to the shaft of the gear reducer which turns only one revolution

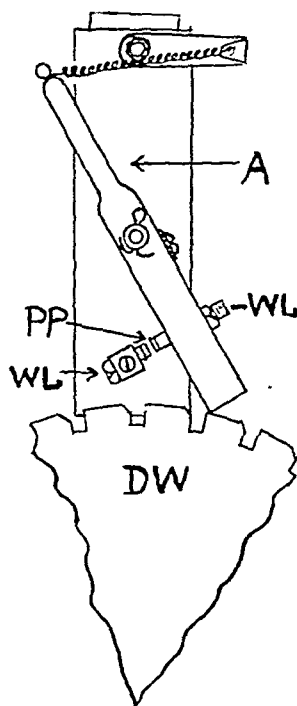


Fig. 2.—Sketch of current "breaker." A, pivoted arm of "breaker" held by tension spring; PP, opposed platinum points; WL, wire leads from pivoted and fixed arm; DW, notched drum wheel.

in a minute. An ordinary gear wheel with teeth or sprockets or a plain wheel with either notches (Fig. 1) or removable pins placed at regular intervals radially or at right angles along the circumference may be used. For producing second intervals with a two-second pause each minute, a wheel with sixty teeth is selected and one of these teeth is removed. Other minute-fraction time intervals may be provided by attaching additional drum wheels with an appropriate number of teeth.

D. The Current "Breaker."—This device can easily be made from two pieces of spring brass or constructed as shown in Fig. 2. Platinum or other

*Described on page 184, Catalogue 50, No. LA 900, Boston Gear Works, North Quincy, Mass.

metallic contact points are placed opposite each other on the inside of these metal pieces. When the pivoted arm of the "breaker" is either raised or lowered, depending on whether it is operated by teeth or notches, contact is made and the circuit momentarily closed. A current sufficient to operate a large number of signal magnets can be sent through these contact points. Very satisfactory "breakers" can also be made from discarded phonejacks which are obtainable for a few cents from almost any radio supply store.

MODIFICATIONS

When time intervals additional to seconds and a two second pause each minute are desired, other properly constructed drum wheels must be attached to

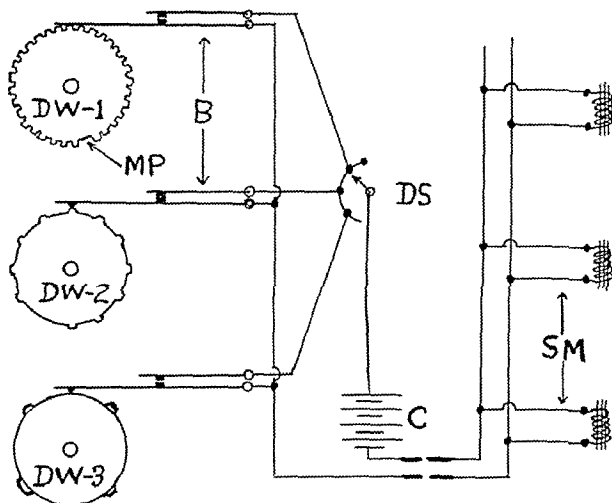


Fig. 3—Diagram showing electrical connections necessary when three drum wheels are used on same shaft. DW-1 drum wheel equipped to provide intervals of seconds and minutes. DW-2 drum wheel providing five second intervals. DW-3 drum wheel providing fifteen second intervals. MP two second pause to indicate minute interval. DS dial switch. C dry cells. SM signal magnets.

the reducer shaft. In such case it is suggested that notched wheels be used as these require less work to make than would be the case if numerous teeth had to be filed off gear or sprocket wheels. Sprocket wheels are preferable to ordinary gear wheels because the sprockets allow the pivoted "breaker" arm to be raised and lowered more rapidly, thereby producing a sharper time curve on the kymograph drum. When several drum wheels are attached to the same reducer shaft, a sliding bar may be used for the "breaker" although it is more convenient to install individual "breakers" for each wheel. With this latter arrangement any

particular drum wheel providing the desired time interval may be switched into the circuit with the signal magnets simply by the turn of a dial as shown in Fig. 3.

Fig. 1 is a sketch of a timing device we have assembled and used with entirely satisfactory results for the past year. The motor is a one-sixth horse power, 1800 R.P.M., 60 cycle synchronous model. It was obtained secondhand from an electrical shop at a cost of \$11.00. Our university machine shop supplied the gears, constructed the "breaker" and frame, and assembled the device for another \$11.00. From our experience we believe that similar devices can easily and cheaply be assembled and that they will be found entirely satisfactory and dependable. It is our opinion that no other timing device on the market today can compare with this new apparatus as to accuracy and positiveness of operation, as well as to simplicity.

AN IMPROVED ELECTROPOLYGRAPH*

RUSSELL A. WAUD, M.D., PH.D., LONDON, CANADA

THE instrument to be described was designed to record simultaneously on photographic paper any two of the following: electrocardiogram, heart sounds, arterial or venous pulse, or apex beat. In one of my previous papers¹ an ink polygraph employing a microphone was described, by which tracings of the arterial and venous pulse were recorded in much greater amplitude than with other types of ink polygraphs previously employed. In another paper by me² a heart sound amplifier employing radio valves was described. The present instrument is a combination of the above instruments with considerable improvements and with the addition of a high gain radio valve amplifier and oscillograph by means of which electrocardiograms three times the amplitude of those ordinarily obtained may be made without distortion.

Fig. 1 is a semidiagrammatic representation of the instrument.

VENOUS PULSE AND APEX BEAT UNIT

In order to record the venous pulse or apex beat, an ordinary receiving tambour is placed over the pulsating area. A rubber tube connects this tambour with a second tambour in which the usual rubber membrane is replaced by a permanent thin metal diaphragm. The metal diaphragm is in firm contact with the button of an ordinary carbon or crystal type microphone and at no time during the operation of the instrument can it be seen to move even when viewed with a microscope. Thus movements over the vessel or heart are made to vary an electric current without the introduction of a vibrating membrane.

*From the Department of Pharmacology, University of Western Ontario Medical School.
Received for publication, July 3, 1935.

The deflecting coils of a galvanometer are in series with the microphone. Thus a beam of light reflected from the galvanometer is made to record the pulse on photographic paper. In order to prevent the constant current always present in the microphone circuit from destroying the sensitivity of the galvanometer by deflecting its mirror to one side, a bucking battery and rheostat are placed across the terminals of the galvanometer. By means of the rheostat the constant current of the microphone circuit flowing through the deflecting coils

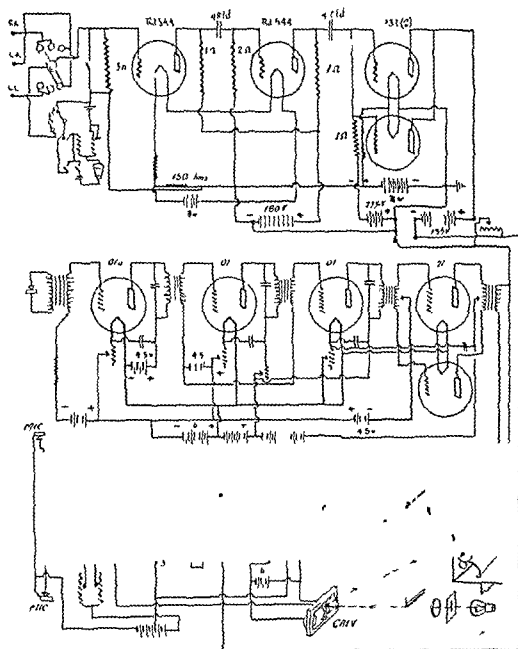


Fig. 1—A schematic diagram of the wiring arrangements of the instrument

can be neutralized by that of the bucking battery. This also serves as an efficient sensitivity control. At times it is an advantage to use a transformer in the circuit. For this purpose a multiple switch is included so that at will the current which ordinarily passes through the microphone can be made to go through the primary of a transformer, while the secondary current operates the galvanometer of the oscillograph. This has the effect of producing more definite end points in the tracing which it is hoped may prove of clinical value.

When recording the apex beat the same method is used as with the venous pulse

ARTERIAL PULSE UNIT

Because of the difficulty of initial adjustment and maintenance of a tambour over the radial artery the conventional wrist tambour is not employed. Instead, a blood pressure cuff is applied to the arm and connected by rubber tubing to a metal tambour and microphone similar to that used when recording the venous pulse. After inflating the cuff above that of the patient's diastolic pressure, the metal tambour is brought in firm contact with the microphone button by means of a micrometer screw. Once applied this continues to operate satisfactorily even with considerable movement of the patient's arm, so that the undivided attention of the operator can be directed to holding the venous receiving tambour.

HEART SOUND UNIT

In recording heart sounds either an electromagnetic or a crystal type microphone is applied to the chest wall. The current generated in the microphone is fed into a high gain radio valve amplifier. The output of the amplifier passes into a loud speaker in which the horn has been replaced by a rubber tube which in turn leads into a Bowle's multiple stethoscope. Condensers of varying values placed across the output serve to suppress room noises and at the same time to intensify certain murmurs. By means of a switch this output current can be made to pass through one of the galvanometers and thus be recorded on photographic paper with the venous or arterial pulse or the electrocardiogram.

ELECTROCARDIOGRAPH UNIT

In recording the electrocardiogram an amplifier of the ordinary resistance coupled type is used to obtain sufficient current to operate the galvanometer of one oscillograph. The condensers are of large capacity to take care of the lowest frequencies occurring in the electrocardiogram. A multiple switch allows shifting from lead to lead. The conventional galvanometer, resistances and standard cell are included for purposes of standardization of the amplifier. In order to get rid of alternating current from outside power lines, which is so often troublesome in electrocardiographic work, a 250,000 ohm rheostat is included in the input so that the offending current may be neutralized by means of a similar current out of phase. The output of the amplifier is connected to the deflecting coils of one of the galvanometers.

THE OPTICAL SYSTEM

Light is obtained from three automobile headlight bulbs, which are supplied with current from an eight volt storage battery. A switch is attached to the knob which operates the camera, so that during standardization and adjustment of the light beams six volts only are allowed on the filaments, while during the actual taking of the record, eight volts are used. This insures ample light even when the camera is operating at maximum speed. Each of the two galvanometers is supplied with a separate source of light as is also the time marker.

The light, after passing through a narrow slit, is condensed by a spherical lens on the mirror of the galvanometer, from the mirror it is reflected into the camera where it is again condensed by the usual cylindrical lens, and the image sharpened by a narrow slit

THE TIME MARKER

The most satisfactory timing device was found to be that in which a spring motor is used to rotate a slotted disk. A modified phonograph motor serves very well for this purpose. This type has the advantage over the electrical in that there is no electrical field being set up which at times may disturb the amplifier. The timing has been found to be very accurate and may be checked at any time by the ordinary stroboscopic method. To the shaft of the phonograph motor is attached a disk with 25 slots cut in its edge, every fifth slot is larger than the others. A beam of light emerging from a slit in the lamp house passes through the revolving disc and by means of a cylindrical lens is focused on the camera lens. Thus intervals of one fifth and one twenty fifth of a second are photographed on the tracings.

THE CAMERA

The camera is designed to use bromide paper 80 mm wide and 50 meters long. The paper is unwound from the unexposed paper box and drawn past the lens by means of an electrically driven friction roller, and is fed into a large removable box, the sliding door of which is supplied with a cutting edge. To load the camera the unexposed paper box is removed and taken to the dark room. The camera is operated by a six volt electric motor which is suspended on springs so as to prevent any vibrations reaching other parts of the instrument. The reduction in speed from the motor to the camera is obtained by means of a spiral gear.

THE GALVANOMETERS

The author is indebted to Mr G. Wootton of the Department of Physics for the design of the galvanometers and to Mr F. W. Pye for their construction. The size and resistance of the coils, damping, properties of the iron, linear response and avoidance of hysteresis, have all been carefully worked out. A minute piece of sheet iron is suspended in a magnetic field produced by a large double solenoid carrying current from a six-volt battery. Two small deflecting coils at right angles to that of the solenoid carry the current to be recorded. Changes in this current cause changes in the magnetic field controlling the piece of iron. A small mirror attached to the iron moves the beam of light across the opening of the camera.

The optical systems, galvanometers, time marker and camera are all housed in a light tight box under a bakelite panel. In order that the light beams may be seen by the operator, the upper portion of the beam is reflected by a mirror on a ground glass screen which is mounted in the bakelite panel.

DISCUSSION

Graphic methods of investigating the heart and circulation are now recognized as producing valuable information concerning diagnosis, prognosis, and

treatment. Without these methods our powers of perception are limited and *imperfect observations are liable to lead to erroneous deductions.* The electrocardiograph is now an instrument of common use and has become an essential part of the equipment of every hospital. However, the polygraph, which Sir James Mackenzie valued so highly, has fallen into disuse. This is due to some extent to the fact that part of the information obtained by means of the polygraph is contained in the electrocardiogram. The difficulties met with in the operation of the ink polygraph and the maintenance of fresh rubber tambours, together with the small and indefinite tracings with which one must so often be satisfied, are, however, the greatest factors in preventing a wider clinical use of the instrument. The pulse unit of the instrument described here is designed to obviate these difficulties. The employment of microphones and electrical amplification allows the replacement of rubber tambours with metal ones, and the recording of large sharply defined tracings in patients in which the venous

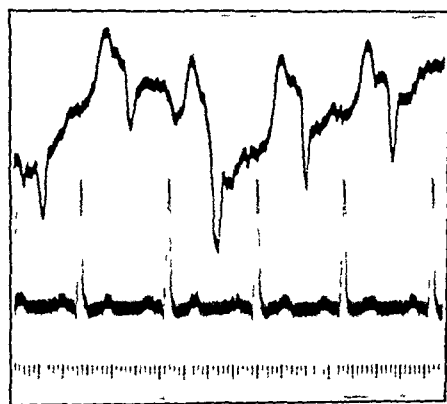


Fig. 2.—A simultaneous tracing of the venous pulse and the electrocardiogram made with the instrument.

pulse is scarcely perceptible. The use of the blood pressure cuff in place of the conventional wrist tambour gets rid of the difficult adjustments ordinarily encountered in recording the arterial pulse.

A high gain electrocardiograph amplifier and a galvanometer capable of responding to high frequencies are included in this instrument because Reid and Caldwell³ have shown that more valuable information can be obtained from electrocardiograms when a true record of greater amplitude than that of the conventional size is obtained. It allows a quantitative rather than just a qualitative analysis of the tracings.

The instrument is enclosed in a wooden case and in order to be portable is mounted on wheels.

Fig. 2 shows a simultaneous tracing of the venous pulse and electrocardiogram made with the instrument.

SUMMARY

A polygraph is described which will record simultaneously on photographic paper any two of the following: electrocardiogram, heart sounds, arterial or venous pulse or apex beat.

These tracings are much greater in amplitude than those obtained with the ordinary apparatus

Rubber tambours have been replaced with metal ones, and a blood pressure cuff is used in place of the tedious wrist tambour

By means of a switch, a transformer, which greatly increases the definition of the tracings, can be introduced into the venous pulse circuit

The author is indebted to Messrs H Higgins, instrument maker, F H Coates, technician, and H Sheppard, technician, for their services and help in constructing the instrument

REFERENCES

- 1 Waud, R A An Electric Polygraph J A M A 82 1263 1924
- 2 Waud, R A A Heart Sound Amplifier J LAB & CLIN MED 16 624, 1931
- 3 Reid W D, and Caldwell, S H Research in Electrocardiography Ann Int Med 7 369, 1933

MUSCLE NERVE STIMULATING ACCESSORY FOR HARVARD KYMOGRAPHS*

O G HARVE AND C LARI BUTTS BALTIMORE, MD

THE device described herein is a compact electrical unit, which is operated by the kymograph mechanism and can be substituted for the driven member of the clutch of any standard Harvard instrument. It is easily set in place without disturbing any of the essential functions or the structure of the kymograph. It can be quickly set to deliver either "make" or "break" shocks or both, at any of the commonly desired intervals of time, and at any predetermined position on the drum. However, if superimposition of one record upon another is desired, as is sometimes the case in comparative phase work, such as the effect of temperature or fatigue upon the phases of a contraction cycle, it is desirable to mount the kymograph and stand carrying the muscle levers and signal magnets upon a common base as illustrated (Fig 1) or use an iron stand with a heavy base. Obviously for these purposes the relationship of stand to kymograph must remain constant.

Those muscle nerve experiments which can be performed with a satisfactory degree of accuracy without difficulty or any great amount of manual dexterity upon the part of the operator are

- 1 A study of the phases of a simple muscle contraction (Make or break stimuli or both for comparison)
- 2 Summation (Using either make break or break make combination)
- 3 Comparative study of the effects of repeated stimulation upon the phases of the contraction cycle

*From the Department of Physiology, University of Maryland School of Medicine
Received for publication July 6 1935

4. The effects of temperature change or degrees of fatigue upon the height and duration of the contraction.

The value of this unit for class work appears to be twofold. In the first place, the student is not lost in a maze of complicated and completely new apparatus. In the second, the results are much more accurate than the average

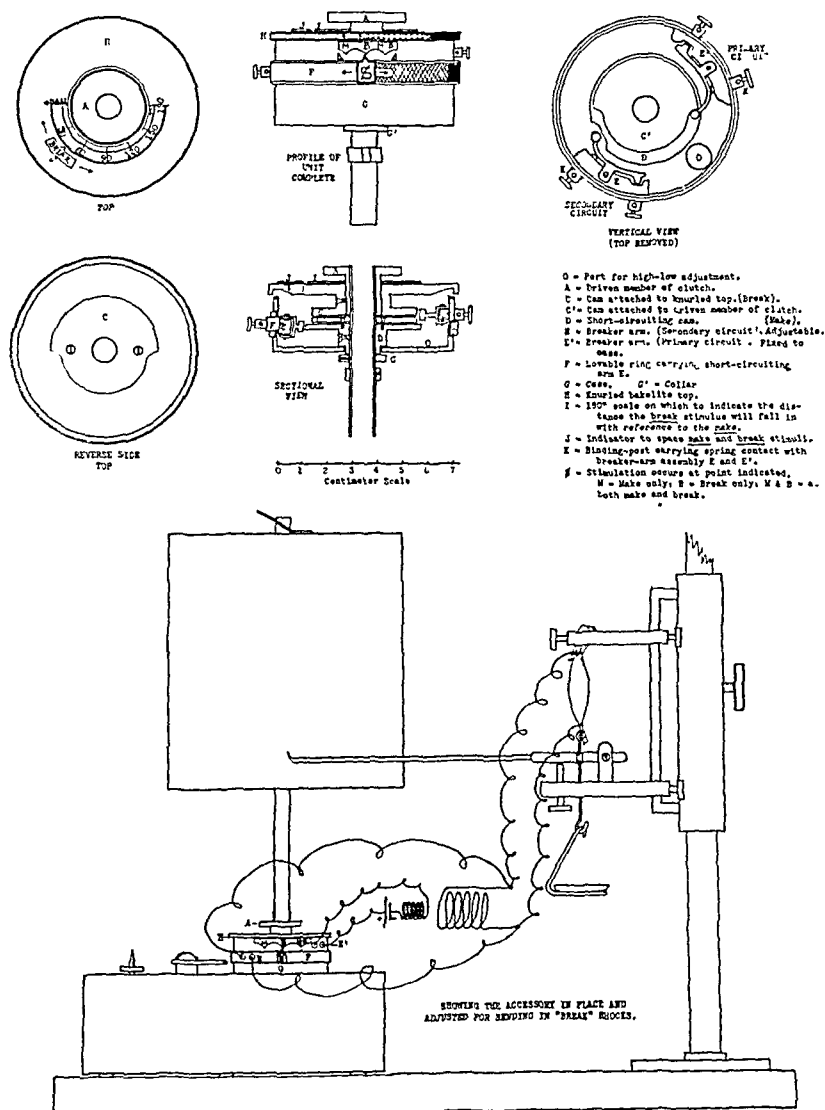


Fig. 1.—Showing structural details of the unit, and the suggested arrangement for its use. (See text for details.)

student could hope to obtain by the manually controlled methods. In practice we have found both factors to play a part in the reaction of the student to the experiment, there being more interest in the result and less anxiety concerning the set-up.

It makes possible also the duplication, in some manner, of those records commonly found in the textbooks of physiology. *A*, *B*, *C*, and *D* in Fig. 2 are records of the effects of temperature change, fatigue, and repeated stimulation respectively, upon the phases of an isotonic twitch of the frog's gastrocnemius muscle. Fig. 2, *E* represents the accuracy with which contraction may be duplicated and superimposed. This curve contains 20 perfectly superimposed contractions.

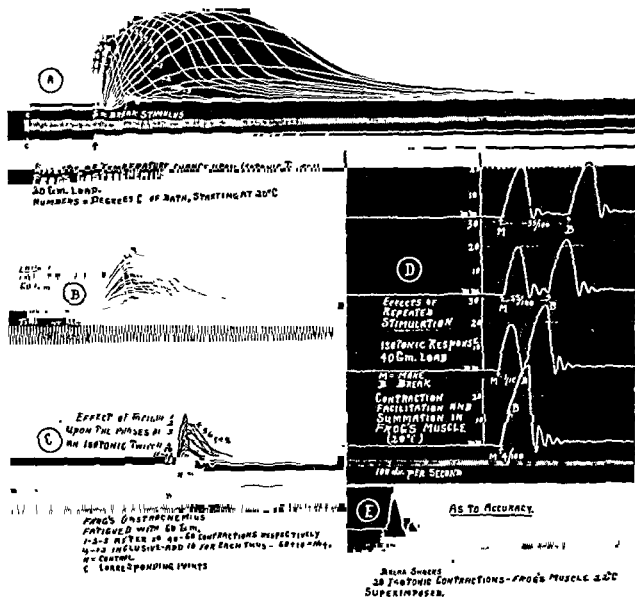


FIG. 2.—Showing how muscle responses may be superimposed *A*, in the study of temperature changes, *B*, in the study of the effects of previous work. *E* is the superimposition of 20 isotonic contractions made as a check of the accuracy of the drum speed and the ability of the unit to duplicate stimulation and record the result. *D* illustrates how "make" and "break" shocks may be used to demonstrate contraction facilitation due to a previous stimulus, and how the interval of time between them may be gradually decreased to show various degrees of summation.

Structural details: The unit consists essentially of 5 units or assemblies as follows:

1. *A case*, which is made from a section of brass tube $1\frac{1}{4}$ inches long having a $\frac{1}{8}$ inch wall fitted with a disc cut from $\frac{1}{16}$ inch brass for a bottom. This makes it into a cup. This cup has three major openings in it—2 in the bottom: one, for the center shaft and the other (Fig. 1, *O*), for the high-low shift unit; 1 in the side, a long slot through which the adjustable breaker arm assembly (*E*) moves.

There are also 2 small insulated holes in the side for carrying the binding posts (*K*) of the fixed breaker arm assembly (*E'*). The case is fitted outside with the necessary index figures ("M" for make, "B" for break) and a movable band (*F*) carrying also the index figure (\$), indicating the point of stimulation, and one of the breaker arm assemblies (*E*).

2. *A cover (H)*, made of bakelite, $\frac{3}{16}$ inch thick, carries on top an index figure marked "Break." On the reverse side and spaced $\frac{3}{8}$ inch from its under-surface is a cam *C*, made of bakelite, $\frac{1}{16}$ inch thick, which is cut exactly opposite to cam *C'* and so adjusted that when the index figure *J* points to the "make" arrow on the 180° scale (*I*), cams *C* and *C'* lying over each other make a perfect disc upon the edge of which the breaker arm of assembly *E'* rides in neutral, thus delivering neither "make" nor "break" shocks. As the cover (*H*) carrying cam *C* is rotated counterclockwise a given distance, a depression of that extent on the edge of cam *C'* is exposed, and permits the breaker arm to fall into it as cam *C'* passes before it, thus making contact in the primary circuit (a make stimulus). As it emerges from the depression, the circuit will be broken, thus giving a "break" stimulus.

3. *The driven member of the clutch*: All exposed parts of this unit are exactly like the member furnished with the kymograph, except where the drum contacts it; here the table is not flat, but hollowed out to give a more rigid contact than usual. This member also carries the brass scale *I*, which, while not an integral part of the unit, is finally firmly attached thereto, and rotates with it.

Inside the unit it will be seen that this member has been lengthened to carry a number of units. Cams *C'* and *D* are firmly attached, while cam *C* is in contact with it and driven by friction carrying with it in rotation also the bakelite cover *H*. All members attached to this unit rotate between the breaker arm assemblies *E* and *E'*, thus making and breaking the primary and secondary circuits appropriately as the index figures on the scales indicate. The timing of cams *C'* and *D* for "make" shocks is illustrated in the vertical view. The interval between "make" and "break" shocks is set by rotating index figure *J* away from the "make" arrow the desired distance and by setting index figure on ring *F* to *M* + *B*. When "make" or "break" shocks alone are desired all that is necessary is to rotate ring *F* to the appropriate index letter.

4. *The breaker arm assemblies*: These are simple metal toggle arms mounted on bakelite blocks so adjusted that both binding posts will be in contact with the arm for a closed circuit and in contact with only one for open circuit. They are activated by the rotating cams.

5. *The retaining collar*: *G'* is a retaining collar to hold the assembly intact and to maintain the proper relations of the parts within, during storage of the unit and while setting it in place.

DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

SICKLE CELL Standardizing Method Beck J S P and Hertz C S Am J Clin Path 5 325, 1935

A test tube method for the production and demonstration of sickle cells is described. Every phase of the test is easily controlled and standardized.

The results of 100 tests by this method are compared with the results of the sealed smear method in the same group at the same time.

An incidence of 13 per cent is reported for the sickle cell trait in 100 negroes.

When the sickle cell trait exists, 77 per cent or more of the erythrocytes may show sickling by the tube method. By the slide method the trait may not be demonstrated at all in many cases.

The method follows:

Apparatus and Reagents

1 Small test tube, 4 x 1 cm

2 Physiologic saline citrate. This may be prepared by mixing equal quantities of normal saline and 3 per cent sodium citrate.

3 Paraffin oil

4 Saline formalin fixative. Add 0.85 gm of sodium chloride to 100 cc of 10 per cent neutral formalin.

5 Glass slides, cover glasses, lancet to prick finger, three small bottles and three small drawn glass pipettes equipped with rubber nipples, and a microscope.

The saline citrate, the paraffin oil and the formalin are to be kept in separate bottles, stoppered and labelled. One pipette is to be used for handling the saline citrate solution, one for the paraffin oil and one for the formalin. Mark these pipettes appropriately to cause a trace of formalin in the blood prior to sickling may prevent the deformity. Before starting the test, place from 0.2 to 0.5 cc of saline citrate in the tube to receive the fresh blood.

Procedure—Clean and prick the finger as in collecting blood for a count. Wipe the finger dry and collect one or two drops of blood in the test tube containing the saline citrate. Invert to mix. Cover with sufficient oil to make a layer 1 cm thick. Make sure no bubbles of air are under the oil. Let the preparation stand at room temperature for twenty-four hours, then introduce 0.2 to 0.5 cc of the formalin solution beneath the oil layer by means of the formalin pipette. Thoroughly mix by forcing the liquids in and out of the pipette several times. Do not break up the oil layer for fear of letting air in too soon. Two or three minutes or more should be allowed for fixation. After this period the suspension is mixed again with the formalin pipette to insure a uniform distribution of cells. Remove a few drops from the tube, wipe away the excess oil from the tip, and place a drop on a glass slide. Cover and examine. The percentage is calculated in the manner of the differential leucocyte count.

If it is desired to measure the blood and the solutions accurately, it may be done with a hemoglobin pipette for the blood and 1 cc pipettes graduated into tenths, for the solutions. It will be found that 20 cmm of blood in the solutions described above make a very satisfactory cell suspension for microscopic observation.

Permanent preparations are made from the suspension on cover glasses like an ordinary fresh blood film. The smears are dried in air and fixed by flame. Blood cells treated with formalin are not well tinted in Wright's stain. Excellent stains may be made by using the hematoxylin-eosin technique, but if it is desired to stain only the erythrocytes, a

simpler method consists of staining with basic fuchsin or some similar dye. To use fuchsin, flood the smear with a 1 per cent aqueous solution for fifteen to thirty seconds. Pour off, wash in water, and differentiate by flooding with 95 per cent alcohol for thirty to sixty seconds. Destaining in alcohol is to be controlled microscopically before mounting. If the cells are too pale, restain and differentiate less. When the staining is satisfactory, wash in water, blot, dry, and mount in balsam.

MENINGITIS, Tuberculous, Age Distribution and Pathogenesis of in Children, Siegel, M.
Am. Rev. Tuberc. 32: 196, 1935.

An analysis was made of the age distribution of 445 cases of tuberculous meningitis among 742 children who died of tuberculosis at Josef-Kinderspital, Mount Sinai Hospital and Sea View Hospital. In addition, a similar analysis was made of approximately 3,000 cases of tuberculous meningitis reported in the literature in the past thirty years.

Of the total cases in childhood, there were 57 per cent in the first three years, 26 per cent in the next three years, and 17 per cent between six and twelve years of age. The greatest number of cases occurred between the ages of nine and fifteen months.

No undoubted case of tuberculous meningitis was reported in infants who died under two months of age of either acquired or congenital tuberculosis. There were a few cases of tuberculous meningitis in the third month. At this age, the percentage of cases of lethal tuberculosis with meningitis was low (7.9 per cent). After the third month, the incidence of tuberculous meningitis in infants who died of tuberculosis increased markedly, reaching 33 per cent in the fourth month and 50 per cent in the sixth month. The percentage gradually increased in older infants and reached its maximum (about 80 per cent) in the third and fourth years.

There were no cases of tuberculous meningitis in 68 cases of acute meningitis in infants below two months of age. About 90 per cent of these 68 cases were caused by the streptococcus, colon bacillus, pneumococcus, and meningococcus.

Additional data are presented to show that tuberculous bacilleemia is the precursor, but not the direct cause, of meningitis, which may eventually result from extension of a local tuberculous focus into the subarachnoid space or ventricles.

The incidence of tuberculous meningitis in lethal tuberculosis, as reported from various hospitals, depends largely on the type of tuberculous case admitted to these hospitals and on the duration of hospitalization.

BREAST TUMORS, Roentgen Diagnosis of, Ritvo, M., Butler, P. F., and O'Neil, E. E.
J. A. M. A. 105: 343, 1935.

Roentgen examination affords an easy and reliable method of studying the female breast. Neoplasms of the breast and other pathologic processes may be visualized. The changes incidental to menstruation, pregnancy and the menopause are also demonstrable.

The roentgen examination requires no special preparation of the patient and causes no pain or discomfort.

Cheate and Cutler's classification of mazoplasia and cystiphorous desquamative epithelial hyperplasia is used to replace "chronic mastitis" and "cystic mastitis," these terms being unsatisfactory in the light of recent studies. The authors suggest the name cystoplasia to denote the condition of cystiphorous desquamative epithelial hyperplasia.

They do not believe at present that the early stages of malignancy or beginning malignant degeneration in formerly benign tumors are demonstrable on the roentgenogram.

Tumor masses can be outlined and the character and extent of the lesion determined. The existence of a neoplasm may be shown by roentgen examination before it can be definitely diagnosed by clinical means.

Glands in the axilla and extension of malignant growth to the ribs are demonstrable on the roentgenogram.

Roentgen studies greatly lessen the need of diagnostic operation and repeated palpation of the breast and give information of value in the diagnosis, prognosis and treatment of lesions of the breast.

LEUKOCYTES, Filament, Non Filament Count in Appendicitis in Children, Mills, S D
Am J Dis Child 50 36, 1935

From an analysis of 41 cases of appendicitis among children, the author would place in order of their reliability as aids in diagnosis (1) an increase in the total leucocyte count, (2) an increase in the number of neutrophiles and (3) an increase in the number of nonfilamented neutrophiles with a nonfilamented filamented cell ratio exceeding 1

Certain points in regard to the filamented nonfilamented cell count seem worthy of mention. In modifications of the well established Schilling count, it has been simplified to the point of eliminating consideration of toxic changes in the neutrophiles, which are often of greater significance than the number of nonfilamented neutrophiles. The presence of these toxic changes in the neutrophiles is of definite prognostic import.

The presence of very immature leucocytes in the course of a case of appendicitis is important evidence of the demand being made on the bone marrow. Immaturity in the leucocytes extending as far as the promyelocyte stage is sometimes seen in cases of acute appendicitis. The nonfilamented cell count provides no means of showing how immature the nonfilamented forms may be.

The nonfilamented cell count may increase in advance of the rise in total leucocytes or neutrophiles. An increase in any or all of these forms should warn one of the possibility of extension of the infection.

The prognostic value of the nonfilamented cell count is perhaps its greatest usefulness. In acute appendicitis, an initial high nonfilamented cell count means that the outlook is bad. With the total percentage of neutrophiles higher than 90 and with less than 10 per cent of these filamented forms, the prognosis is grave. A falling nonfilamented cell count, or a nonfilamented filamented cell ratio of less than 1 is a good sign, as are also the reappearance of eosinophiles and monocytes and the disappearance of neutrophiles of toxic type from the peripheral circulation.

The nonfilamented cell count is of value in the diagnosis of appendicitis since it parallels the neutrophile count during the course of the infection. When considered with the total leucocyte count and the percentage of neutrophiles the nonfilamented cell count is an important diagnostic aid, but it is not reliable alone in a sufficient number of cases to be of the same diagnostic worth as the total leucocyte count and the percentage of neutrophiles.

ENCEPHALITIS, Fulminating Hemorrhagic, Levinson, A., and Saphir, O
Am J M Sc 1 42, 1935

Fulminating hemorrhagic encephalitis is characterized by sudden onset, great respiratory difficulty, coma, and rapid death. The course of the disease is very short and death may sometimes be sudden. The cerebrospinal fluid may be hemorrhagic, but is usually clear. It may have an increase in cells, or may show no changes. The greater the meningeal involvement, the more pronounced the cerebrospinal fluid changes. Histologically, the brain reveals a predominance of a hemorrhagic exudate in addition to perivascularly arranged lymphocytes and neutrophiles. In one instance, fulminating hemorrhagic encephalitis was found superimposed on an old encephalitis. In children, a generalized enlargement of the lymphadenoid apparatus is usually present. The sudden death due to fulminating hemorrhagic encephalitis may be regarded as an example of "sudden death from natural causes," in Kohlsko's definition of the term.

SCARLET FEVER, Dick Test and Blood Agar Cultures as Aids in the Diagnosis of Scarlet Fever, Gasul, B M., and Rhoads, P S
Am J Dis Child 49 603, 1935

Two hundred and seventy three white patients who were admitted to the Cook County Contagious Disease Hospital between June 20, 1932, and April 7, 1933, with a diagnosis of scarlet fever were given a Dick test soon after admission.

Of 30 patients tested on the first day of the disease, 17, or 56.7 per cent, showed a negative reaction the next day; and of 204 patients tested at the end of the first week of the disease, 141, or 69.1 per cent, showed a negative reaction.

Of the total number of patients who were tested, 264 were discharged with a final diagnosis of scarlet fever and 253 of these, or 95.8 per cent, showed a negative reaction before discharge from the hospital. Eleven, or 4.2 per cent, did not show a negative reaction after an average stay of 25.8 days in the hospital.

For 81 patients for whom the final diagnosis was scarlet fever, blood agar cultures were made. The hemolytic streptococcus was present in every case.

Seven of the 9 patients for whom the final diagnosis was not scarlet fever had negative cultures for the hemolytic streptococcus. Cultures were not made for the other 2 patients.

The diagnosis of scarlet fever cannot depend on a single Dick test. A persistently positive reaction to the Dick test during the whole course of the disease throws great doubt on the correctness of the diagnosis of scarlet fever, while a positive reaction at the beginning of the disease together with a negative reaction during the course of the disease is confirmatory evidence that the disease is scarlet fever.

According to the authors' experience, cultures negative for hemolytic streptococci, properly taken at the beginning of the disease on a properly prepared medium, and properly interpreted, offer strong evidence against the correctness of a diagnosis of scarlet fever. A positive culture, however, in the presence of the other clinical findings of scarlet fever confirms the diagnosis.

TISSUE, Improved (Paraffin Section) Method for the Dopa Reaction, Becker, S. W., Prayer, L. L., and Thatcher, H. Arch. Dermat. & Syph. 31: 190, 1935.

Freshly excised tissue, from 3 to 5 mm. in thickness, was immersed in the solution of di-oxy-phenylalanine prepared according to the simplified technic of Laidlaw and Blackberg. The solution was changed after one-half hour, since Laidlaw and Blackberg called attention to the fact that some tissues are sufficiently acid to alter the pH of the solution. The first solution washes out the objectionable substances and the reaction proceeds unhindered in the fresh solution. The pH of the dopa solutions varied from 7.35 to 7.8. Bloch stated that 7.35 is the optimum pH. The solution containing the piece of tissue was kept in the incubator at 37° C. for from twelve to fifteen hours. Within this time the tissue and the solution became black. A period of this length is necessary for penetration and does not produce a great deal of nonspecific blackening of the inner portion of the block.

During the experiments, control frozen sections were placed in the same solution, allowed to remain in the incubator for from three to four hours, washed in water and mounted on glass slides. The collagen of the dermis acts as a medium for mounting. The tissue used for frozen sections was fixed in a 5 per cent solution of formaldehyde for from one to two hours to aid in the cutting.

The block of tissue was removed from the dopa solution and treated with various fixing solutions. Alcohol and formaldehyde did not prove satisfactory, and the best results were obtained by fixing the block in Bouin's solution, according to the method of Masson, for from forty-eight to seventy-two hours. It was then carried through alcohol washings and toluene and embedded in paraffin containing 5 per cent beeswax. Sections of from 5 to 6 microns in thickness were cut. The paraffin was removed with toluene or xylene; the xylene was removed with alcohol, and the sections were taken from the 70 per cent alcohol and placed in water. They were allowed to remain in running water for one hour, at which time the trinitrophenol was completely washed out and the collagen appeared to be pale gray. After this the sections were counterstained by a good staining method. If a quick, simple stain was desired, brazilin was used. For careful cytologic study, the Masson trichrome stain (iron hematoxylin, acid fuchsin-ponceau de xylydine and aniline blue) was used. It is advisable to stain the protoplasm rather lightly with the acid fuchsin-ponceau de xylydine mixture in order to facilitate identification of the dopa-positive cells.

ALLERGIC DERMATOSES, Wise, F., and Wolf, J J A M A 104 1489, 1935

The following classification is proposed

I Eczematous Reactions

- 1 Site of shock tissue epidermis
- 2 Reaction time twenty four or more hours
- 3 Characteristic lesion spongiosis and intrapidermal vesicle
- 4 Causative substances frequently simple chemicals or products of fungi
- 5 Type of test, patch test

II Tuberculin Trichophyton Type Reactions

(not infrequently combined with eczematous responses)

- 1 Shock tissue upper cutis, cutis
- 2 Reaction time from twenty four to forty eight hours or more
- 3 Characteristic lesion lymphocyte and later epithelioid cell infiltration
- 4 Causative substances usually microorganisms, bacterial or fungous products (allergy of infection)
- 5 Test intracutaneous (forty eight hours)

III Urticarial Reactions

- 1 Shock tissue upper cutis (blood vessels)
- 2 Reaction time from ten to thirty minutes
- 3 Characteristic lesion edema extravasation of fluid and eosinophiles (wheal)
- 4 Causative substances foods inhalants, products of microorganisms
- 5 Test intracutaneous scratch or indirect (immediate wheal and flare)

IV Miscellaneous Reactions (drug eruptions "ids" etc)

- 1 Shock tissue deep cutis, cutis epidermis, follicles
- 2 Time from minutes to days
- 3 Lesions (a) nodules, (b) fixed polychromatic areas (c) multiform dermatoses and follicular lesions, etc
- 4 Substances drugs, microorganisms
- 5 Test usually inconclusive (sometimes the patch, Moro or intradermal test is of value)

SYPHILIS, Factors Conditioning the Transmission of Syphilis by Blood Transfusion
Morgan, U J Am J M Sc 129 808, 1935

The transmission of syphilis by blood transfusion in the reported cases in the literature has been manifested in the recipient by the development of the acute secondary stage of the disease within from one to three and one half months after the transfusion. Our present knowledge does not allow of a diagnosis of "transfusion syphilis" in the absence of such developments in the recipient.

Syphilis is obviously transmissible by blood transfusion when, and only when, the virus is present in the donor's blood. Spirochetemia is known to occur only during the early stages of the infection before the development of latency or during pregnancy in the chronic disease. Our present knowledge does not allow of a diagnosis of "transfusion syphilis" in the absence of these stages of syphilitic infection in the donor.

ANAEROBIC BACILLI Infections by Gas Forming, Reeves J R J A M A 104 526, 1935

Approximately 30 per cent of cases reported and treated as *Clostridium welchii* gas gangrene are caused by other anaerobic organisms.

This 30 per cent of cases should be classed as putrefactive gangrene and treated conservatively by systemic supportive treatment, debridement and irrigations. *Clostridium welchii* antitoxin is not indicated and may be injurious.

Putrefactive gangrene is likely to appear in patients past the age of fifty years who are constitutionally below normal. Cases appear notably in patients with circulatory failure, arteriosclerosis, thromboangiitis obliterans, and diabetes mellitus.

The direct cause for putrefactive gaseous lesions in the muscles of patients whose condition has been described may be bruises, burns, simple fractures, parenteral administration of drugs and solutions, and circulatory failure.

Putrefactive gas-forming anaerobic soil bacteria contaminate food at all times. It seems apparent that bacteria of this type are frequently present in the organs and muscles of the aged individual.

Diagnosis of *Clostridium welchii* infection cannot be based on direct smear, the rabbit inoculation test, or Nerb's test.

Diagnosis, for clinical purposes, of the presence of *Clostridium welchii* can be made in experienced hands by capsule stains. However, the only positive identification is by the use of anaerobic cultural methods and related criteria.

Patients suffering from wounds, particularly of muscle tissue, which have been contaminated with soil or street dirt, should be given gas gangrene antitoxin prophylactically and in addition should receive débridement and irrigations. If toxic symptoms develop, radical débridement, continuous irrigations and therapeutic serum are indicated without delay.

LEUKEMIA, Heterophil Antibody Test in, Weinstein, G. L., and Fitzhugh, T., Jr. Am. J. M. Sc. 190: 106, 1935.

The heterophile antibody titer in the sera of 16 cases of leucemia was found to be uniformly and repeatedly at a low level (Zone 1) regardless of the stage and type of the disease. One case of acute myelogenous leucemia was found to be in Zone 2. This patient, however, had received 28 blood transfusions. This constant finding may be of value in ruling out the diagnosis of leucemia in any case in which a high heterophile antibody titer is found.

A low or normal titer of heterophile antibody was found also in 3 cases of Hodgkin's disease, 5 of lymphosarcoma, 5 of polycythemia vera, 4 of agranulocytic angina, and a number of miscellaneous cases including typhoid fever, simple adenitis, syphilis, tuberculosis, anemia, etc.

A high titer (Zone 3) was found in serum sickness and acute infectious mononucleosis, thus confirming the reports of Paul, Bunnell, Davidsohn, and others.

The parenteral administration of horse serum did not produce a rise in the heterophile antibody titer in 5 cases of chronic lymphatic leucemia. This finding is in accord with previous evidence.

A similar failure of increase of heterophile antibody titer following horse serum injections was found in 1 case of "atypical" Hodgkin's disease and 2 of lymphosarcoma, suggesting the possibility of a biologic relationship of these conditions to lymphatic leucemia.

The parenteral administration of horse serum in 3 cases of chronic myelogenous leucemia produced a marked rise in heterophile antibody titer similar to that occurring in nonleucemic individuals. This finding is not in accord with previous evidence, and suggests the possibility of a real biologic difference between myelogenous leucemia on the one hand, and the lymphatic group on the other hand.

EOSINOPHILIA, in Scarlet Fever, Freedman, S. Am. J. Dis. Child. 49: 1256, 1935.

The course of the eosinophile count in 85 cases of scarlet fever has been traced and the presence of primary and secondary eosinophilia has been demonstrated. The nature of the phenomenon has been discussed and the view has been expressed that the formation of antitoxin and the presence of free antitoxin in the blood constitute the most logical explanation of the eosinophilia.

REVIEWS

Books and Monographs for Review should be sent direct to the Editor,
Dr Warren T. Vaughan, Professional Building, Richmond, Va

Post Mortems and Morbid Anatomy*

THIS, the third edition of a useful book, has been extensively revised and rewritten, notably in the sections on endocarditis, pulmonary tuberculosis, gastric ulceration, nephritis and the nephropathies, and gastric ulcerations

Many new illustrations have been added and the volume may be regarded as a safe, comprehensive, and authoritative guide

The Foot†

THE importance of diseases of the foot, even those of comparative triviality, as a cause of social discomfort and industrial incapacity is widely recognized

The more marked lesions receive the attention of orthopedists and are the subject of lectures to the student, but the more trivial lesions, though quite often of import, are likely to be overlooked both by the student and the practitioner. It is to remedy this gap that this book has been written, and well written by one whose experience has fitted him for the task

The book is clearly written, well illustrated, and should prove a useful addition to the physician's library

The Hair and Scalp‡

AS THE author remarks in her preface, while diseases of the scalp and hair are too often regarded as minor maladies hardly deserving of serious consideration by the physician, to those who suffer from them, particularly women, they may be matters of utmost importance

It is, perhaps, this not uncommon attitude toward these conditions which results in the trial, first, of the innumerable remedies so glowingly described in advertisements that when the physician is finally consulted in desperation the condition may present, as, indeed, even the most common diseases of the scalp and hair not infrequently present, a puzzling problem. And, unfortunately, they are also problems which, either by reason of lack of interest or information, the physician is often but ill equipped to handle

These facts form the underlying *raison d'être* of this book in which Dr Savill has attempted, and with no small measure of success, to discuss in an intelligent, scientific, and quite comprehensive manner the diseases to which the scalp and hair may be subject

The manner of presentation is that familiar to the readers of Savill's Clinical Medicine. The chief symptom is first noted and followed by a list of possible causes which are then reviewed and differentiated. The disease in question is fully described and the methods applicable to its treatment and control fully discussed

*Post Mortems and Morbid Anatomy. By Theodore Shennan M.D. Professor of Pathology University of Aberdeen. Cloth pp 716 240 figures. William Wood & Co. Baltimore Md.

†The Foot. By Norman C. Lake Senior Surgeon and Lecturer on Surgery, Charing Cross Hospital London etc. Cloth pp 330 and 95 figures. William Wood & Co. Baltimore Md.

‡The Hair and Scalp with a chapter on Hirsuties. By Agnes Savill A.M. M.D. (Glasg.) Consulting Physician to Fitzroy Square Skin Hospital etc. Cloth pp 288 and 53 figures. William Wood & Co., Baltimore

Manson's Tropical Diseases*

TIME was when books concerned with the recognition and management of tropical diseases were of interest principally to those who, by reason of geographical location, were likely to encounter them.

The rapidity and extent of modern transportation facilities, however, as well as the intermingling of the races of the world during the World War, have to a large extent nullified the former geographical distribution of diseases so that while there are still diseases more or less limited to tropical countries, many heretofore so classified may now be seen in new locations. It has become necessary, therefore, for the physician in general to have, at least, access to reference texts on this subject than which few are better known than that of Manson.

The six years which have elapsed since the last edition of this well known work have seen many advances which are included in the present revision, so much so that the title is now more a matter of convenience than exactitude since the book is very comprehensive in scope.

The diversified field included in this edition is indicated by the eleven sections into which it is divided: I Fevers, II Vitamin Deficiency Diseases, III Abdominal Diseases, IV Infective Granulomatous Diseases, V Diseases of the Central Nervous System, VI Tropical Venereal Diseases, VII Tropical Skin Diseases, VIII Local Diseases, IX Animal Parasites and Associated Diseases, X Diseases Due to Poisons, including Snake Bites, etc., XI Technique. An appendix is devoted to medical zoology and laboratory methods.

A feature of this edition is the large amount of space given to discussions of the clinical aspects of the diseases considered and also to the methods now available for their treatment.

The book is profusely and excellently illustrated. An extensive index renders the contents readily accessible.

This volume may be recommended as a comprehensive and authoritative reference text which without doubt will achieve as heretofore, a deservedly wide circulation.

Recent Advances in Cardiology†

THE third edition of this useful book has been entirely rewritten and contains much new material in addition to the thorough revision required to keep in line with the advances in cardiology since the previous edition four years ago.

As heretofore, the main theme of the book is the importance of health and efficiency of the cardiac muscle, and the arrangement of the material is such that all the sequelae of cardiac inefficiency are discussed in a logical and orderly manner.

Those familiar with previous editions of this text will not need to be reminded of its eminently practical plan and the excellent manner in which it is carried out.

For the student, the specialist, and especially for the practitioner at large, this book continues to be a valuable, comprehensive and authoritative text which can be highly commended.

For and Against Doctors‡

IT MAY occur to some, perhaps, that the doctor and his works have been the subject of an extraordinary amount of discussion in recent times, but the literature of the world witnesses that these have always been of great interest to the laity.

*Manson's Tropical Diseases. A Manual of the Diseases of Warm Climate. Edited by Philip H. Manson-Bahr. El 16. Cloth. 1092 pages. 381 text figures. 22 color plates. 15 half-tone plates. 6 maps and 38 charts. William Wood & Co. Baltimore, Md.

†Recent Advances in Cardiology. By Terence, J. M. D., Physician, King's Hospital and Curtis Bain, Physician, Harrowgate General Hospital. El 3. Cloth. 370 pages. 14 plates and 85 text figures. P. Blakiston's Son & Co. Philadelphia, Pa.

‡For and Against Doctors. An Anthology Compiled by Robert Hutchison and G. M. Wauchope. Cloth. with 168 pages. William Wood & Co. Baltimore, Md.

If the pages of the world's writers contain instances of extravagant censure, these may be counterbalanced by equally extravagant praise, facts utilized by Dr. Hutchison in his MacAllister lecture for 1934.

The great and general interest thus aroused led to the preparation of the present anthology which embraces an extensive selection culled from many sources as the following headings indicate: Proverbs, The Ancients, Medieval, Fifteenth to Eighteenth Century, The Moderns, Retrospect.

An index renders the contents of the book readily accessible.

This little volume should be a welcome and valuable addition to the physician's library and should be of particular interest to the medical writer in search of an apt reference and quotation.

Modern Criminal Investigation*

WRITTEN primarily for policemen, detectives, and peace officers in general, this book nevertheless will be of great interest to lawyers, students of criminal investigation, police surgeons, pathologists, physicians, and also to the intelligent layman.

It is well written, comprehensive, and authoritative, and furnishes a complete picture of the varied procedures now called into play in the modern investigation of crime.

The reader of the "mystery stories" of today is familiar with the fact that many technical procedures culled from various arts and sciences now form a part of the modern police department and that many specialists are now an important part of the personnel. In this volume he will see just what is done and how it is done and, without doubt, will be surprised at the advances which have been made and the varied procedures now utilized.

The story thus told is not only informative but exceedingly interesting and the book can be recommended as an excellent discussion of, perhaps, the most important problem now confronting any community.

Experimental Bacteriology†

IN VIEW of the well-deserved popularity of "Kolle and Hetsch," long a familiar German reference text, the appearance of this first English text by Professor Eyre will undoubtedly meet with a most cordial reception.

It may be said with some confidence that there are few, if any, texts covering this subject as comprehensively as this one. Certainly none present it in quite the same manner.

Throughout the two volumes, in which not only the diseases peculiar to man, but also those seen in animals are discussed, there is a steady insistence upon the importance of animal experiment in every phase of bacteriologic investigation. It is for this reason, perhaps, that this work has maintained its commanding position in the laboratory field in which, in many ways, it serves as the bacteriologist's vade mecum.

It is not, however, a book for the laboratory worker or the bacteriologist alone, for the sections on specific diseases contain a wealth of material of interest and value to all those concerned with the management, treatment, or control of disease.

Without question, the English translation of this standard text will achieve and retain an equal popularity with its German predecessors.

*Modern Criminal Investigation. By Harry ... D.Sc. Head of The Institute of Police Science, University of Stockholm, Sweden, and Deputy Chief Inspector, New York City Police Department, and Dean of thousands of drawings and 31 plates. Funk and Wagnalls Co., Cloth, 461 pages, numer-

†Experimental Bacteriology: Its Applications to the Diagnosis, Epidemiology, and Immunology of Infectious Diseases. By Dr. W. Kolle, Director, Institute for Experimental Therapy etc., Frankfurt, and Dr. H. Hetsch, Professor at the Institute for Experimental Therapy, Frankfurt. English translation of seventh revised German edition by John Eyre, M.D., Professor of Bacteriology, University of London. Cloth, Vol. I, pp. 592; 49 plates and 79 figures. Vol. II, 62 plates, 120 figures, The Macmillan Co., New York.

The Journal of Laboratory and Clinical Medicine

Vol. 21

JUNE, 1936

No. 9

CLINICAL AND EXPERIMENTAL

HEMOGRAPHY IN THE DIAGNOSIS OF APPENDICITIS BASED ON 500 CASES*

WALTER J. CROCKER, V. M. D., and ELEANOR H. VALENTINE, M. D.,
PHILADELPHIA, PA.

IN RECENT years there has been an attempt to obtain more information from the leucocyte count and the stained blood smear than is afforded by the simple Ehrlich differential count. A number of methods for classifying the neutrophils have been offered, among the best known being those of Schilling and of Arneth.

The Schilling method of dividing the neutrophils into four groups based on their maturity, i. e., myelocytes, juveniles, stabs and segmenters is in common use, being recognized as a more complete and valuable diagnostic aid than the conventional Ehrlich method and more simple than the complicated Arneth system.

It is logical that the Schilling principle should be applied to the differentiation of various types of appendicitis and we have, therefore, employed a modification of the Schilling hemogram.

Kohl uses only a part of Schilling's classification of neutrophils to distinguish four degrees of appendicitis which he does not describe. He bases his diagnosis on the total percentage of immature neutrophils (myelocytes + juveniles + stabs) which Schilling calls "shift cells," e. g.,

SHIFT CELLS	APPENDICITIS
11-20%	First degree
25-45%	Second degree
45-60%	Third degree
60 and above %+	Fourth degree

*From the Division of Clinical Pathology of the Laboratories of the Philadelphia General Hospital.

Received for publication August 27, 1935

In appendicitis, as in all infections, it is not sufficient to consider the shift cells alone in making a diagnosis from the hemogram. This one item becomes more important when considered in its relation to the total leucocyte count, the percentage of segmenters, total neutrophils, lymphocytes, monocytes, etc.

We believe that the entire hemogram, when carefully analyzed and considered with the total number of shift cells, affords the most accurate differentiation. It is possible, we believe, to recognize the various stages of appendicitis from the most quiescent, fibrotic state to the most active, diffuse inflammatory process.

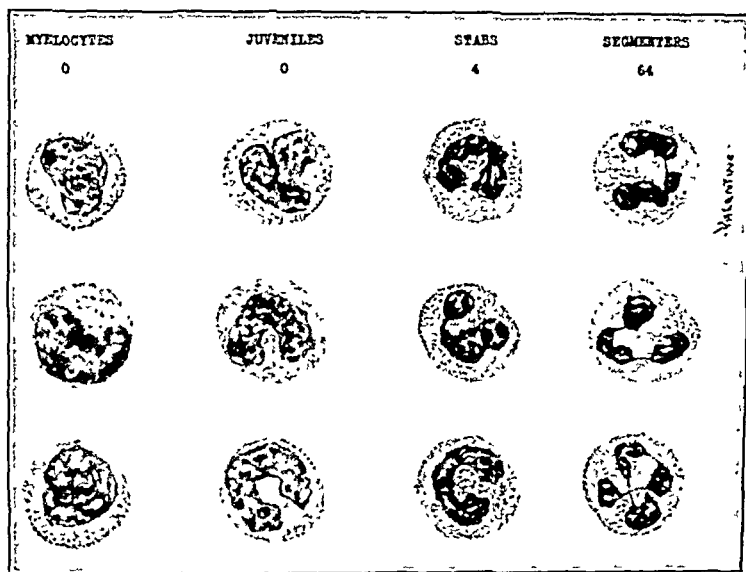


Fig. 1.—Staining with Wright's stain and buffer solution. (1) Wright's stain one minute. Cover the slide with stain, 10 to 15 drops. (Cells fixed by the methyl alcohol and staining begun.) (2) Buffer solution three minutes. Add an equal quantity of buffer solution to the Wright's stain on the slide, 20 to 30 drops. (Staining completed.) (3) Wash in distilled water. (4) Blot. (5) Decolorize in 95 per cent alcohol one second to remove stain deposit. (6) Wash in distilled water immediately until the slide stops streaking. This must be done quickly to stop the decolorizing action of the alcohol. (7) Blot.

Wright's stain, Biosal Products Co., Rochester, N. Y., 1 tablet to 10 c.c. of absolute methyl alcohol C. P.

Buffer Solution pH 6.45.

Stock Sol. 1. 28 c.c.

Stock Sol. 11. 72 c.c.

Dist. H₂O 900 c.c.

Stock Sol. 1. Na₂PHO₄.2H₂O (11.87 grams dissolved in 1,000 c.c. distilled water)
Na₂HPO₄.2H₂O is obtained by spreading Na₂HPO₄.12H₂O in a thin layer and drying in the air for two weeks.

Stock Sol. 1. Na₂HPO₄.2H₂O (11.87 grams dissolved in 1,000 c.c. distilled water)

HEMOGRAPHY

We have modified the Schilling hemogram by differentiating the lymphocytes on the basis of their maturity, in addition to the neutrophils, and by adding several indices. For detailed information our previous paper* may be consulted.

*Crocker and Valentine: Hemography in Diagnosis, Prognosis and Treatment, Based on 6000 Schilling Hemograms, J. LAB. & CLIN. MED. 20: 172, 1934.

Normally there are in the peripheral blood approximately 5,000 neutrophiles per cubic millimeter distributed as follows according to Schilling

myelocytes	juveniles	stabs	segmenters	total neutrophiles
0	0	4	64	68

Schilling calls myelocytes, juveniles and stabs, "shift cells". Except for 4 to 10 per cent stabs these cells are absent from the peripheral blood during health, but may appear in varying numbers during infections and intoxications. An increase in the number of shift cells with a decrease in segmenters is called a shift to the left, and indicates that the severity of the infection is increasing. A decrease in the number of shift cells with an increase in segmenters, is a shift to the right and indicates subsidence of an acute or subacute infection or may possibly be due to a low grade chronic intoxication.

The quantity of the shift is expressed by the Schilling index and the multiple index. The Schilling index is the ratio of the total shift cells to segmenters, e.g.,

M	J	St	Sg	
0	0	4	64	4/64 or 1/16, normal Schilling index, no shift
M	J	St	Sg	
6	18	48	24	72/24 or Schilling index of 3, wide left shift

The "multiple index" is the Schilling index multiplied by 16 or the Schilling index expressed somewhat differently. The normal multiple index is therefore $\frac{1}{16}$ times 16 or 1. While these indices have the same relative value they are expressed differently for convenience in analyzing the hemogram. The Schilling index gives the ratio of immature to mature neutrophiles. The multiple index shows the number of times the shift is greater than normal and therefore may indicate the degrees of infection or intoxication.

The quality of the left shift is determined by the type of shift cells appearing in the peripheral blood. Myelocytes, juveniles, and stabs are found in varying numbers depending on the severity and type of the infection or toxic agent. These cells are found in the bone marrow normally but are probably not sufficiently mature to pass the threshold into the peripheral blood. Increasing toxic depression of this threshold permits the escape of the immature neutrophiles from the bone marrow in the order of their maturity as though they were formed in layers. The reserve segmenters pass the threshold first and are soon depleted, then the stabs, next the juveniles, and finally the myelocytes, thus extending the qualitative shift progressively to the left.

The qualitative shift must be considered with the quantitative shift as indicated by the Schilling index, since the latter gives no indication of the individual types of shift cells which are being allowed to pass the threshold. For a quantitative shift or Schilling index of 3, the qualitative shift might be

M	J	St	Sg	
0	0	60	20	= 60/20 or SHI 3
		or		
M	J	St	Sg	
6	24	30	20	= 60/20 or SHI 3

The quantitative shift is the same in both hemograms, but the quality of the shift is different in each. The first is a simple degenerative or stab left shift.

The latter is a leucemoid or myelocytic left shift with myelocytes and juveniles present in addition to stabs. It indicates a much greater depression of the threshold and a more severe intoxication. This information cannot be obtained from the quantitative shift or Schilling index alone.

The lethal index is qualitative in character. It is arbitrarily determined by obtaining the ratio of all the myelocytes, one-half the juveniles or one-eighth of the stabs to the segmenters. When it reaches one or over and the multiple index is 100 or above, the patient is certainly extremely ill, so ill that a fatal outcome is common.

By the term "threshold" we mean that mechanism which qualitatively controls the maturation and release of the cells which pass from the bone marrow into the peripheral blood during infections or intoxications.

The term "regenerative power" refers to the power of the bone marrow to increase the production of neutrophils under toxic stimulation. In some cases "toxic pressure" may be so great that this ability is decreased or lost and production of neutrophils is less than normal.

The hemogram is a horizontal tabulation of the various important items in the blood picture.

The shiftograph is a vertical graph of the Schilling indices of a chart and indicates the extent of the quantitative shift to the left or right, in relation to the qualitative shift. The direction and extent of the graph reveals the course of illness of the patient which can be roughly estimated at a glance.

The shiftogram we refer to as a chart of serial hemograms connected by a shiftograph, showing the entire course of the disease. Analysis from day to day may show extension or subsidence, convalescence or complication and frequently signs of approaching death. It may be a guide to medical and surgical treatment and probably constitutes a valuable indicator in determining improvement or retrogression of a patient under many forms of therapy in routine or research problems.

DESCRIPTION AND DIFFERENTIATION

Five hundred cases of appendicitis were studied clinically and by hemogram, and the following degrees could be differentiated:

First Degree or Chronic Fibrous Appendicitis.—With vague clinical symptoms and a history of recurrent attacks the hemogram of chronic fibrous appendicitis presents quite constant characteristics.

The white cell count usually remains within the normal limits of 5,000 to 10,000 per c.mm. The percentage of neutrophils ranges from 40 to 70, total shift cells 10 to 35, lymphocytes 20 to 40, monocytes 1 to 15, eosinophiles 0 to 6, basophiles 0 to 5, lethal index $\frac{1}{40}$ to $\frac{1}{2}$, multiple index 4 to 16, Schilling index $\frac{1}{4}$ to 1, Freyfeld's toxic granules 0, Doehle's inclusion bodies 0, and the hemogram conforms to Type 5 A.

The neutrophilic left shift is confined almost exclusively to stabs. It is always slight and with the neutropenia may point to an insidious absorption of toxin or a low grade intoxication. The lymphocytosis with few monocytes and eosinophiles indicates an attempt at immunohealing or repair by proliferation of connective tissue.

a process of atrophy than inflammation; subacute appendicitis, a beginning acute exacerbation of chronic fibrous appendicitis or a late subsiding acute

No. of hemogram	Diagnosis	WBC per mm	Lethal index	Multiple index	Schilling index	Myelocytes	Juveniles	Stabs	Segmenters	Total neutrophils	Lymphoblasts	Large lymphs	Intermediate lymphs	Small lymphs	Total lymphs	Monocytes	Eosinophiles	Basophiles	Toxic granules	Inclusion bodies	Type of hemogram
		WBC	LI	MI	ShI	M	J	St	Seg	TN	Lb	L	S	TL	M	E	B	IG	IB	T	
1	Ch hyperplastic App, glands and follicles	7500	1/128	5	1/6	0	0	4	64	68	0	5	1	20	26	4	0	1	00	00	4
2	Ch hyperplastic App, glands and follicles	15500	1/21	16	1/3	0	0	15	42	57	0	6	0	30	36	6	0	0	00	00	4
3	Ch hyperplastic App, glands and follicles	12000	1/8	17	1.0	0	0	30	30	60	0	0	0	36	36	4	0	0	00	00	4
4	Ch hyperplastic App, glands, follicles, lymph infil	11100	1/7	17	1.1	0	0	35	30	65	0	0	0	33	33	0	0	0	00	00	4
5	Neurogenic App (?) hypertrophy nerve plexuses	13000	1/9	16	1.0	0	0	35	37	72	0	0	0	22	22	4	2	0	00	00	4
6	Appendicitis	12400	1/10	16	1.0	0	0	30	36	66	3	5	0	24	33	1	0	0	00	00	4
7	Appendicitis	13000	1/22	5	1/3	0	0	18	45	63	3	3	0	18	24	9	3	1	00	00	4
8	Subacute App, Exacerb of ch	12400	1/17	8	1/2	0	0	25	50	75	0	0	0	20	20	5	0	0	00	00	4
9	Subacute App, subsiding acute	10800	1/10	12	3/4	0	0	34	40	74	0	5	0	2	8	14	2	2	00	00	4
10	Subacute App, subsiding, ac ex of ch	13700	1/14	10	2/3	0	0	20	35	55	0	20	0	15	35	10	0	0	00	00	4
	Pyo-appendix	10000	1/25	5	1/3	0	0	18	57	75	0	2	0	9	11	24	0	0	00	00	4

Chart 2.—Second degree appendicitis, illustrating hemograms which are similar throughout but which represent subacute or chronic pathologic processes in the appendix, e.g., atrophy, hypertrophy, degeneration, inflammation, confined sterile pus or mucus and various combinations of these changes.

appendicitis; and hydro-appendix or pyo-appendix, when complete fibrotic stenosis of the proximal end of the appendix causes filling of the structure with mucus or pus which is probably sterile or contains an organism of very low virulence.

With vague symptoms and a history of recurrent attacks the hemogram of second degree appendicitis is quite constant for this group of conditions. The white blood cell count is moderately high and ranges from 10,000 to 15,000 per c mm. The percentage of neutrophils ranges from 50 to 75, total shift cells 15 to 35, lymphocytes 10 to 35, monocytes 1 to 15, eosinophiles 0 to 4, basophiles 0 to 3, lethal index $\frac{1}{2}$ - to $\frac{1}{4}$, multiple index 5 to 17, Schilling index $\frac{1}{4}$ to 1.1, Fretwell's toxic granules 0, Doehle's inclusion bodies 0, and the hemogram conforms to Type 4.

Differentiation of second degree and third degree appendicitis. Confusion is not probable because of the acute symptoms and high white cell count and neutrophilia of the third degree or acute suppurative early gangrenous appendicitis, even though the low indices and low total shift cells may be like those of second degree appendicitis.

Number of hemogram		WBC per cmm		Lethal index		Multiple index		Schilling index		Myelocytes		Juveniles		Stabs		Segmenters		Total neutrophiles		Lymphoblasts		Large lymphs		Intermediate lymphs		Small lymphs		Total lymphs		Monocytes		Eosinophiles		Basophiles		Toxic granules		Inclusion bodies		Type of hemogram	
No	WBC 7500	LI 1/128	MI 1	ShI 1/16	M 0	J 0	St 4	Sg 64	TN 68	Lb 0	L 51	I 1	S 20	TL 26	M 4	M 2	B 0	TG 00	IB 00	T	normals																				
1	16300	1/38	3	1/5	0	0	16	76	92	2	4	0	2	3	0	0	0	00	00	1																					
2	18200	1/10	12	3/4	0	0	34	42	70	0	4	0	4	3	10	0	0	00	00	1																					
3	18500	1/20	5	1/3	0	0	22	62	84	0	5	0	8	13	3	0	0	00	00	1																					
4	19200	1/22	6	2/5	0	0	26	68	94	0	2	0	0	2	4	0	0	00	00	1																					
5	21000	1/22	6	2/5	0	0	25	68	93	0	3	0	4	7	0	0	0	00	00	1																					
6	22150	1/14	9	4/7	0	0	32	58	90	0	0	0	4	4	6	0	0	00	00	1																					
7	23000	1/18	8	1/2	0	0	26	54	80	0	6	0	6	12	2	4	2	00	00	1																					
8	25300	1/19	8	1/2	0	0	27	57	84	0	3	0	0	9	7	0	0	00	00	1																					
9	26200	1/10	10	2/3	0	0	36	54	90	0	0	0	4	4	6	0	0	00	00	1																					
10	31500	1/13	9	3/5	0	0	32	52	84	0	2	0	2	4	12	0	0	00	00	1																					

Chart 3—Third degree or acute suppurative early gangrenous appendicitis illustrating high leucocytosis and neutrophilia but only a slight degenerative left shift (Frozen threshold)

Differentiation of second degree and fourth to eighth degrees of appendicitis is made easy by the higher white cell count, neutrophilia, wider shift, total shift cells over 35, and higher indices of the latter.

Third Degree or Acute Suppurative Early Gangrenous Appendicitis.—With typical symptoms of acute appendicitis this hemogram is remarkably constant. The white cell count is always high, approximating 18,000 per c mm with high neutrophilia. It is peculiar in that the left shift is always slight, with Schilling index a fraction, multiple index close to 16, and the total shift cells approximately 20. This inactivity of the maturation and release mechanism of the bone marrow, after becoming slightly depressed, we call a "frozen threshold." The area of gangrene may vary from the size of a pea to one third of the appendix.

Toxin from this gangrenous area seems to stimulate the regenerative power of the bone marrow to produce large numbers of neutrophiles but at the same time possibly inhibits or “freezes” the threshold at a slightly depressed level so that only a few shift cells escape into the peripheral blood. To indicate the presence of confined, infected pus, 35 or more total shift cells are usually found, yet this hemogram, with as few as 15 shift cells, consistently indicates the condition to be an acute suppurative early gangrenous process when the clinical symptoms are typically those of an acute appendicitis.

The leucocyte count of third degree appendicitis ranges from 15,000 to 30,000 per c.mm., the neutrophiles from 75 to 95, total shift cells 15 to 35, lymphocytes 2 to 25, monocytes 1 to 15, eosinophiles 0 to 4, basophiles 0 to 2 per cent, lethal

Number of hemogram		WBC per cmm		Lethal index		Multiple index		Schilling index		Myelocytes		Juveniles		Stabs		Segmenters		Total neutrophiles		Lymphoblasts		Large lymphs		Intermediate lymphs		Small lymphs		Total lymphs		Monocytes		Eosinophiles		Basophiles		Toxic granules		Inclusion bodies		Type of hemogram	
No	WBC 7500	LI 1/128	MI 1	ShI 1/16	M 0	J 0	St 4	Seg 64	TN 68	Lb 0	L 5	I 1	S 20	Tl 36	M 4	E 2	B 0	TG 00	IB 00	T	normals																				
1	8000	1/5	25	1.6	0	0	42	25	67	0	15	0	12	27	6	0	0	00	00	2																					
2	8400	1/5	24	1.5	0	0	50	20	70	0	12	0	8	20	6	4	0	00	00	2																					
3	9800	1/4	36	2.3	0	0	53	23	76	0	0	0	23	23	1	0	0	00	00	2																					
4	11100	1/8	16	1.0	0	0	36	36	72	0	0	0	16	16	12	0	0	00	00	2																					
5	11600	1/7	19	1.2	0	0	40	35	75	0	8	0	10	18	7	0	0	00	00	2																					
6	11900	1/6	20	1.3	0	0	40	30	70	0	22	0	0	22	4	2	2	00	00	2																					
7	12700	1/5	25	1.6	0	0	42	25	67	0	5	0	20	25	0	8	0	00	00	2																					
8	12800	1/8	19	1.2	0	3	35	32	70	0	10	0	7	17	12	1	0	00	00	2																					
9	13000	1/3	40	2.5	0	0	50	20	70	0	2	0	28	30	0	0	0	00	00	2																					
10	15300	1/6	19	1.2	0	0	42	33	75	0	6	3	6	15	8	2	0	00	00	2																					

Chart 4.—Fourth degree or acute suppurative exacerbation of chronic appendicitis, illustrating variation in white blood cell counts and uniformity of indices and other items of the hemograms.

index $\frac{1}{40}$ to $\frac{1}{10}$, multiple index 3 to 16, Schilling index $\frac{1}{5}$ to 1, Freyfeld's toxic granules 0, Doehle's inclusion bodies 0, and the hemogram is a Type 1.

Differentiation of third degree and the higher degrees of appendicitis is made chiefly on the “frozen threshold” or slight left shift, few shift cells and low indices, while the fourth to eighth degrees show a wide left shift and total shift cells usually over 40 per cent.

Fourth Degree or Acute Suppurative Exacerbation of Chronic Appendicitis.—With an acute attack in recurrent appendicitis this hemogram constantly shows a white cell count approximating 13,000, a neutrophilia close enough to 75 per cent to indicate an acute process, total shift cells usually 40 per cent or above indicating confined pus, while lymphocytes approximately 30 per cent and a few monocytes point to chronicity.

Fourth degree or acute suppurative exacerbation of chronic appendicitis shows a range of white blood cells from 7,000 to 15,000 per c mm, neutrophilia 60 to 75, total shift cells 35 to 60, lymphocytes 20 to 40, monocytes 1 to 15, eosinophiles 1 to 10, basophiles 0 to 3 per cent, lethal index $\frac{1}{8}$ to $\frac{1}{4}$, multiple index 16 to 48, Schilling index 1 to 3, Freyfeld's toxic granules 0, Doehle's inclusion bodies 0, and the hemogram is a Type 2

Fourth degree appendicitis is distinguished from first, second, and third degree processes by its higher indices and total shift cells being over 35, indicating pus. It is not confused with fifth sixth seventh and eighth degrees chiefly because of its high lymphocytes, relatively low neutrophilia and low leucocyte count. In rare instances it may be confused with the sixth degree or acute suppurative appendicitis not ruptured, when the latter is walled off by the omentum

No of hemogram	WBC per cmm	Lethal index	Multiple index	Schilling index	Myelocytes	Juveniles	Stabs	Segmenters	Total neutrophilice	Lymphoblasts	Large lymphs	Intermediate lymphs	Small lymphs	Total lymphs	Monocytes	Eosinophilres	Basophilres	Toxic granules	Inclusion bodies	Type of hemogram
No	WBC 7500	LY 1/128	MI 1	ShI 1/16	M 0	J 0	St 4	Se 64	TN 68	Lb 0	L 5	I 120	S 26	TL 4	M 4	E 2	B 0	TG 00	IB 00	#
1	9100	1/4	35	2.2	0	0	47	25	72	0	3	0.25	28	0	0	0	00	00	2-4	
2	12300	1/5	28	1.8	0	0	36	20	56	0	6	0.10	16	28	0	0	00	00	2	
3	13700	1/6	20	1.3	0	0	43	33	76	0	5	0.11	24	0	0	0	00	00	2	
4	13850	1/5	22	1.4	0	3	45	33	81	0	0	0.15	15	4	0	0	00	00	2	
5	14200	1/3	40	2.5	0	0	50	20	70	0	16	0	16	12	1	1	00	00	2	
6	16100	1/6	19	1.2	0	0	38	30	68	2	6	0	6	14	16	0	0	00	00	2
7	16550	1/5	22	1.4	0	3	36	27	66	3	9	0.12	24	10	0	0	00	00	2	
8	18100	1/6	17	1.1	0	0	45	39	84	0	6	0	6	20	0	0	00	00	2	
9	19300	1/5	24	1.5	0	0	48	32	80	0	6	0.10	16	2	2	0	00	00	2	
10	25100	1/5	20	1.3	0	0	37	28	65	0	0	0.128	28	7	0	0	00	00	2	

normal

Chart 4.—Fifth degree or acute suppurative appendicitis ruptured with walling off pus. Clinical prerequisite: mass in right lower quadrant and symptoms of acute appendicitis. This chart illustrated variation of leucocytosis and uniformity of indices and other items of the hemogram.

or mesentery which may induce an equally high lymphocytosis. Here the history aids in differentiation as fourth degree is recurrent in type and sixth degree shows acute symptoms of a first attack.

Fifth Degree or Acute Suppurative Appendicitis Ruptured, Mass in Right Lower Quadrant, Walling Off—The prerequisites for this hemogramic diagnosis are a mass in the right lower quadrant with symptoms and history of ruptured appendix. The characteristic hemogramic features comprise a comparatively high percentage of lymphocytes and monocytes with a rather low neutrophilia. Total shift cells are over 35 per cent and the white blood cell count usually high but may be as low as 9,000 per c mm. The higher the lymphocytes and monocytes the more advanced is the connective tissue proliferation in the walling off

process. The higher the neutrophilia and lower the lymphocytes and monocytes the less extensive is the walling off process. The percentage of total shift cells being over 35 points to confined pus.

In fifth degree appendicitis the total white cell count ranges from 10,000 to 30,000 per c.mm. In percentage the neutrophiles range from 60 to 90, total shift cells 35 to 60, lymphocytes 5 to 30, monocytes 0 to 30, eosinophiles 0 to 2, lethal index $\frac{1}{8}$ to $\frac{1}{3}$, multiple index 16 to 48, Schilling index 1 to 3, Freyfeld's toxic granules 0, Doehle's inclusion bodies 0, and the hemogram conforms to Type 2, with an occasional Type 4 being seen.

Differentiation of fifth degree and other degrees of appendicitis.—The palpable mass in the right lower quadrant with history of first attack and symptoms of ruptured appendix rule out other forms of appendicitis. Without the symptoms of ruptured appendix but with first attack and palpable mass in the

No.	No. of hemogram	WBC per cmm	Lethal index	Multiple index	Schilling index	Myelocytes	Juveniles	Stabs	Segmenters	Total neutrophiles	Lymphoblasts	Large lymphs	Intermediate lymphs	Small lymphs	Total lymphs	Monocytes	Eosinophiles	Basophiles	Toxic granules	Inclusion bodies	Type of hemogram
No	WBC	LI	MI	SHI	M	J	St	Sg	TH	Lb	L	I	S	TL	M	E	B	TG	IB	T	normals
	7500	1/128	1	1/16	0	0	4	64	68	0	5	1	20	26	4	2	0	0	0	0	
1	7300	1/4	27	1.7	0	0	48	27	75	0	0	0	15	15	10	0	0	00	00	4	
2	10400	1/4	28	1.8	0	0	54	30	84	0	2	0	8	10	6	0	0	00	00	2	
3	11200	1/3	36	2.2	0	0	60	26	86	0	4	0	10	14	0	0	0	00	00	2	
4	14300	1/7	17	1.1	0	0	48	42	90	0	0	0	3	3	7	0	0	00	00	2	
5	15200	1/5	28	1.8	2	2	54	32	90	0	10	0	0	10	0	0	0	00	00	2	
6	16000	1/4	35	2.2	0	0	55	25	80	0	5	0	8	13	7	0	0	00	00	2	
7	17100	1/5	24	1.5	0	0	57	38	95	0	0	0	3	3	2	0	0	00	00	2	
8	19200	1/4	28	1.8	0	0	52	28	80	0	0	0	16	16	4	0	0	00	00	2	
9	20000	1/6	20	1.3	0	0	47	35	82	2	0	0	8	10	8	0	0	00	00	2	
10	26200	1/7	17	1.1	0	0	48	44	92	0	0	0	8	8	0	0	0	00	00	2	

Chart 6.—Sixth degree or acute suppurative appendicitis, not ruptured, illustrating variation in leucocytosis with uniformity of indices and other parts of the hemograms. Total shift cells high but under sixty.

right quadrant this hemogram could indicate acute suppurative appendicitis, not ruptured but walled off by omentum or mesentery. If the history cannot be obtained, the mass in the right lower quadrant is not definite, and the pus is well walled off, inducing high lymphocytes, the fifth degree hemogram may be confused with that of the fourth degree or acute suppurative exacerbation of chronic appendicitis.

Sixth Degree or Acute Suppurative Appendicitis Not Ruptured.—With a history of first attack and acute symptoms of appendicitis this degree is characterized by high neutrophilia, lymphopenia, variable monocytes, total shift cells over 35 per cent, and white cell count usually high.

In sixth degree or acute suppurative appendicitis, not ruptured, the white blood cell count ranges from 7,000 to 30,000 per c.mm., neutrophiles 75 to 95, total

shift cells 35 to 60, lymphocytes 0 to 20, monocytes 0 to 10, eosinophiles 0 to 3, basophiles 0 per cent, lethal index $\frac{1}{8}$ to $\frac{1}{3}$, multiple index 16 to 48, Schilling index 1 to 3, Frickefeld's toxic granules 0, Doehle's inclusion bodies 0, and the hemogram is a Type 2

Differentiation of this degree and seventh degree or acute suppurative appendicitis, ruptured, is based on the total shift cells being over 60 per cent in the latter, together with symptoms of ruptured appendix. Occasionally the sixth degree hemogram will fail, and the appendix be ruptured. In these cases the monocytes are high and range from 10 to 20 per cent, white blood cell count as high as 25,000, total shift cells will be between 50 and 60 per cent, neutrophils approximately 80 per cent, and the multiple index will be above 25. The clinical symptoms and diagnosis, however, are those of ruptured appendix and peritonitis.

No. of hemogram	WBC per cent	Lethal index	Multiple index	Schilling index	Myelocytes	Juveniles	Stabs	Segmenters	Total neutrophiles	Lymphoblasts	Large lymphs	Intermediate lymphs	Small lymphs	Total lymphs	Monocytes	Eosinophiles	Basophiles	Toxic granules	Inclusion bodies	Type of hemogram
No.	WBC 7500	LI 1/128	MI 1	ShI 1/16	M 0	J 0	St 4	Seg 64	TN 68	Lb 0	L 5	I 1	S 20	TL 26	M 4	E 2	B 0	TG 00	IB 00	T
1	6000	1/5	28	1.8	0	0	60	33	93	0	0	0	3	3	4	0	0	00	00	2
2	11300	1/4	35	2.2	0	0	62	28	90	0	0	0	3	3	7	0	0	00	00	2
3	15600	1/5	27	1.7	0	0	60	35	95	0	0	0	5	5	0	0	0	00	00	2
4	17800	2/5	48	3.0	0	0	63	21	84	0	0	0	3	3	12	1	0	00	00	2
5	18400	1/5	32	2.0	2	4	54	32	92	0	6	0	2	8	0	0	0	00	00	2
6	20700	1/4	35	2.2	0	0	60	27	87	0	3	3	3	9	4	0	0	00	00	2
7	23200	1/4	30	1.9	0	0	62	32	94	0	0	0	2	2	4	0	0	00	00	2
8	28500	1/5	28	1.8	0	0	60	34	94	0	0	0	6	6	0	0	0	00	00	2
9	31200	1/5	29	1.8	0	4	56	34	94	0	2	0	2	4	2	0	0	00	00	2
10	33500	1/4	35	2.2	0	0	62	28	90	0	0	0	6	6	4	0	0	00	00	2

normal

Chart 7—Seventh degree or acute suppurative appendicitis, 75 per cent ruptured, 25 per cent ready to rupture illustrating variation in leucocytosis with uniformity of indices and other items of the hemogram. Total shift cells 60 or above.

Seventh Degree or Acute Suppurative Appendicitis, Ruptured or Ready to Rupture—With a history of first attack and symptoms of acute appendicitis the hemogram of this degree is characterized by high neutrophils, lymphopenia, variable monocytes, practically no eosinophiles or basophiles, white blood cell count usually high and shift cells over 60 per cent.

Approximately 75 per cent of appendices showing this hemogram will be found to have ruptured, while 25 per cent will be ready to rupture. The latter show a small perforation or the wall to be so pervious to bacteria that a fibrinous peri-appendicitis and sometimes extensive gangrene have developed. The higher the total shift cells rise above 60 per cent the greater certainty there is that the appendix has ruptured. Nothing definite can be determined, in this regard, by the white blood cell count alone.

In the seventh degree or acute suppurative appendicitis, ruptured or ready to rupture, the white cell count ranges from 6,000 to 35,000 per c.mm., while all other features of the hemogram remain fairly constant for this condition. The neutrophiles range from 80 to 95, total shift cells 60 to 75, lymphocytes 0 to 15, monocytes 0 to 12, eosinophiles 0 to 3, basophiles 0 to 1 per cent, lethal index $\frac{1}{3}$ to $\frac{1}{2}$, multiple index 27 to 64, Schilling index 1.7 to 4, Freyfeld's toxin granules 0, Doehle's inclusion bodies 0, and the hemogram is a Type 2.

Differentiation of the seventh degree or acute suppurative appendicitis, ruptured or ready to rupture, from sixth degree, or acute suppurative appendicitis not ruptured, lies in the total shift cells being over 60 per cent in the former. Seventh degree appendicitis is distinguished hemogramically from the eighth degree or acute suppurative appendicitis, ruptured with diffuse peri-

No. of hemogram		WBC per cmm		Lethal index		Multiple index		Schilling index		Myelocytes		Juveniles		Stabs		Segmenters		Total neutrophiles		Lymphoblasts		Large lymphs		Intermediate lymphs		Small lymphs		Total lymphs		Monocytes		Eosinophiles		Basophiles		Toxic granules %		Inclusion bodies %		Type of hemogram	
No	WBC 7500	LI 1/128	MI 1	ShI 1/16	M 0	J 0	St 4	Seg 64	TN 68	Lb 0	L 5	I 1	S 20	TL 26	M 4	N 4	B 0	TC 00	IB 00	T	normals																				
1	6100	1.8	262	16.4	2	10	70	5	87	2	4	0	2	8	5	0	0	100	20	3																					
2	8000	9/10	140	7.5	0	2	73	10	85	0	10	0	3	13	2	0	0	00	00	2																					
3	8750	1/2	67	4.2	4	14	45	15	78	1	11	1	9	21	1	0	0	00	00	2																					
4	10300	9/10	125	7.8	2	4	72	10	89	0	2	4	6	12	0	0	0	00	00	2																					
5	13900	12.0	1200	75.0	5	23	47	0	75	0	2	0	20	22	3	0	0	50	10	2																					
6	14000	6.0	416	26.0	12	37	30	3	82	0	5	0	10	15	3	0	0	25	6	2																					
7	15800	3.0	368	23.0	3	18	48	3	72	0	3	0	21	24	4	0	0	70	16	2																					
8	16700	3.0	425	26.6	9	6	66	3	84	0	3	0	3	6	10	0	0	100	55	2																					
9	21500	5/8	76	4.8	0	2	76	16	94	0	0	0	6	6	0	0	0	00	00	2																					
10	37100	9/10	101	6.3	0	2	80	13	95	0	2	0	3	5	0	0	0	40	10	2																					

Chart 8.—Eighth degree or acute suppurative appendicitis, ruptured with diffuse peritonitis. White blood cell counts vary. Other features of the hemograms are similar in that they show high neutrophilia, high total shift cells, wide qualitative left shifts, and very high indices which vary with extent of peritonitis or presence of septicemia.

tonitis, by the higher indices of the latter. Furthermore, diffuse peritonitis presents a much wider qualitative left shift, usually showing several myelocytes and juveniles in addition to stabs.

Eighth Degree or Acute Suppurative Appendicitis, Ruptured With Diffuse Peritonitis.—This hemogram is typical of diffuse peritonitis from any cause. The clinical history and symptoms of acute suppurative appendicitis with rupture of the appendix place it in this classification. The chief hemogramic features consist of high indices, high neutrophilia, and wide qualitative left shift with myelocytes and juveniles frequently high.

In the eighth degree or acute suppurative appendicitis, ruptured, with acute diffuse peritonitis, the white blood cell count ranges from 5,000 to 40,000 per c.mm., neutrophiles 75 to 100, total shift cells 75 to 100, lymphocytes 5 to 25,

Schilling, in the *Blood Picture*, page 127, describes the nucleus of the segmenter (segment nuclei neutrophile leucocyte) in these words, "usually has 2 to 5 irregular segments which are connected in a chain of *fine threads*" Cooke is frequently credited with having contributed this distinguishing feature. However, at least one thread of chromatin connecting two segments or a clear open space between two segments should be found to warrant classifying a neutrophile cell as a segmenter.

It is suggested that the study and interpretation of changes in the number, morphology, and combinations of the peripheral white blood cells, in single and sequential hemograms give definite information regarding the character and extent of the underlying general pathology and serve as a valuable guide in determination of the special pathologic process. This method is not simply a different one of doing a "white and differential" but constitutes a new technique for the study of infectious diseases from the standpoint of diagnosis, prognosis and treatment.

From the study of the blood picture we recognize eight degrees of appendicitis with fairly characteristic hemograms for each. Occasionally the hemogramic diagnosis is not consistent with the pathologic condition of the appendix, as illustrated by the shiftogram of a case of necrosis of the appendix. That the apparently normal appendix, in this instance, was responsible for the preoperative clinical and hemogramic diagnosis was proved by the prompt postoperative right shift to recovery, subsidence of symptoms and by examination of the sections.

These hemograms are presented not as definitely diagnostic but as a valuable aid to diagnosis that can be supplied by the clinical laboratory. The limitations of the simple "white cell count and differential," in this respect, have long been recognized.

The term "degree" is used for comparison with the work of Kohl and Schilling. We do not in any way, suggest the substitution of "degree" for the conventional terms now used for the various inflammatory states of the appendix.

The successful use of the Schilling hemogram is, in this field as in others a matter of practice and experience. An effort should be made in every case to check the preoperative diagnosis suggested by the hemogram with the histopathologic diagnosis. This is not so essential when gross examination shows the appendix to be distended with pus, gangrenous or ruptured. Many such findings in our group were not checked by microscopic diagnosis.

If the hemogram is carefully studied, in the light of clinical data, accurate diagnosis may be made in about 90 per cent of cases. This refers to the "degree" of appendicitis, no attempt has been made in this paper to differentiate appendicitis from other pathologic processes.

SUMMARY

We have outlined a laboratory method for the diagnosis of different kinds of appendicitis by a modification of Kohl's method based on Schilling's original work. Hemography is briefly discussed. Eight degrees of appendicitis are described hemogramically and differences pointed out. The diagnostic and prognostic values of the method are discussed.

CONCLUSIONS

1. A more accurate diagnosis of appendicitis can be made by a modified Schilling hemogram than by the use of any other form of white blood cell study. The history and clinical symptoms are, of course, to be considered in any event.

2. It is possible, by hemogram, history and symptoms to distinguish between eight degrees of inflammation or pathologic states of the appendix.

3. Limited white cell differential counts tend to discourage investigation of the blood picture in its relationship to infectious diseases and may close the door to much valuable information.

We express our appreciation to Dr. William Dameshek, Beth Israel Hospital, Boston, for many valuable suggestions. We are deeply grateful to the chiefs of the various wards of the Philadelphia General Hospital for their cooperation and permission to use their cases.

REFERENCES

1. Arneth, J.: Neutrophilen weissen Blutkörperchen bei Infektionskrankheiten, Jena, 1904, G. Fisher.
2. Baum, F.: Importance of Serial Hemograms, *Am. Med.* 35: 348, 1929.
3. Boerner, F.: A Chart and System for Reporting and Recording Blood Examinations, *Am. J. Clin. Path.* 11: 403, 1932.
4. Boyd, W.: Surgical Pathology, Philadelphia, W. B. Saunders Co.
5. Brody, Wm., and Crocker, Walter J.: Specific Immunotransfusion in the Treatment of Septicemia, *J. A. M. A.* 98: 2191, 1932.
6. Crocker, Walter J., and Valentine, E. H.: Hemography in Diagnosis, Prognosis and Treatment Based on 6,000 Schilling Hemograms, *J. LAB. & CLIN. MED.* 20: 172, 1934.
7. Crocker, Walter J., Valentine, E. H., and Brody, Wm.: Hemography-Controlled Non-specific Immunotransfusions in the Treatment of Septicemia and Other Acute Infections, *J. LAB. & CLIN. MED.* 20: 482, 1935.
8. Dameshek, Wm.: Progress in Hematology 1929-1933, *New England M. J.* 210: 531, 1934.
9. Eisenberg, A. H., and Nemens, H. S.: Value of the Schilling Hemogram in Infections; Preliminary Report Based on 3,500 Cases, *Am. J. Surg.* 21: 56, 1933.
10. Fitz-Hugh, T., Jr.: Present Day Modifications of the Arneth Count in Surgical Practice, a Critical Review, *Internat. Surg. Digest.* 15: 195, 1933.
11. Gerard, J. H., and Boerner, F.: The Significance of "Shift to the Left" in Differential Leucocyte Counts and the Nuclear Index as a Means for Interpreting and Recording, *J. LAB. & CLIN. MED.* 16: 300, 1930.
12. Goodale, R. H., and Manning, M. E.: The Schilling Index in Appendicitis, *J. LAB. & CLIN. MED.* 16: 386, 1931.
13. Harkins, H. N.: The Present Status of Blood Examination in the Diagnosis of Surgical Infections With a Study of Twenty-Seven Indices of Infection Reported in the Literature, *Surg. Gynec. Obst.* 59: 48, 1934.
14. Harter, J. S., and Lyons, C.: Surgical Applications of the Schilling Differential Blood Count, *Surg. Gynec. Obst.* 106: 182, 1933.
15. Kotke, Elmer E.: Nonspecific Immunotransfusion in the Treatment of Hemolytic Streptococcus Septicemia, *J. Iowa M. Soc.* 8: 431, 1934.
16. Kohl: Quoted by Schilling, *The Blood Picture*, p. 154.
17. Piney, A.: Recent Advances in Hematology, Philadelphia, P. Blakiston's Son & Co.
18. Prochnow, F.: Die Bedeutung der pathologischen Granulation der weissen Blutkörperchen und des Hamogramms in der Diagnose der akuten Appendicitiden, *Arch. f. klin. Chir.* 166: 160, 1931.
19. Rathmell, Thomas K., and Crocker, Walter J.: The Velocity Factor in Blood Transfusion, *J. LAB. & CLIN. MED.* 19: 1206, 1933.
20. Reznikoff, Paul: Immature White Blood Counts in Infectious Diseases, *J. A. M. A.* 93: 963, 1929.
21. Sabin, F., and Doan C, A.: Bone Marrow as an Organ, *Proc. Soc. Exper. Biol. & Med.* 25: 121, 1927.
22. Stevenson, Ruth: Nonspecific Immunotransfusion in Hemolytic Streptococcus Septicemia, *J. A. M. A.* 100: 100, 1933.

- 23 Schilling, V The Blood Picture, St Louis, 1929, The C V Mosby Co
- 24 Thompson, L D The Schilling Differential Blood Count as an Aid in Surgical Diagnosis, Graham's Surgical Diagnosis, Philadelphia, 1930, W B Saunders Co, p 988
- 25 Warnock, F B A Comparative Study of the Leucocyte Count and Histopathology in Acute Appendicitis, Value of the Schilling Count in Establishing the Diagnosis, Am J Surg 21 47, 1933
- 26 Yaguda, A Studies on Schilling Count in Appendicitis, Am J Clin Path 1 39, 1931
- 27 Carlson, Herbert A, and Wilder, Lucietta The Schilling Hemogram in Appendicitis, Arch Surg 30 325, 1935

THE EFFECT OF OBSTRUCTIVE JAUNDICE ON THE BLOOD PLATELETS OF THE RABBIT*

L V DILL, DURHAM, N C

SINCE Greig Smith¹ mentioned the danger of postoperative hemorrhage in patients with obstructive jaundice, abnormality of several of the elements of the coagulation system has been thought of as a possible explanation of this hemorrhagic tendency. Koechig² found a slight reduction in serum calcium. Kirk and King,³ and Emerson⁴ confirmed these findings, but demonstrated in addition even a relatively greater decrease in diffusible calcium. Walters and Bowler,⁵ Snell, Greene, and Rowntree,⁶ and Zimmerman⁷ observed no decrease in total serum calcium. Gunther and Greenberg⁸ demonstrated a decrease in total serum albumin, causing no decrease in the active or diffusible calcium.

Fibrinogen was found to be maintained at high levels in biliary obstruction experimentally by Moss⁹ and clinically by Gram.¹⁰

Howell¹¹ has postulated that an excess of heparin may be the cause of the delay in clotting. Jones and Minot¹² found no decrease of the blood platelets in a case of catarrhal jaundice, but believed that there was a gradual quantitative increase as the depth of the jaundice decreased. Our own interest in the problem of the hemorrhagic tendency associated with jaundice arose in combination with a case of thrombocytopenic purpura which we had opportunity to study both clinically and at autopsy.

The patient, a white male, aged thirteen, had a clinical history of thrombocytopenic purpura with hemorrhages into the skin and mucous membranes of three weeks' duration. There was slight jaundice that had steadily been decreasing, and the blood showed an indirect van den Bergh reaction. The blood findings were: Hb 8 gm, RBC 2,500,000, WBC 7,500, differential, great predominance of polymorphonuclear cells, platelets 5,000. Autopsy confirmed the clinical impressions of hemorrhages into the skin, mucous membranes, and digestive tract. There were also hemorrhages into the pleurae and the kidney pelvis. The liver showed biliary cirrhosis and marked jaundice. The retroperitoneal and mesenteric lymph node sinuses were filled with blood, the lungs showed an interstitial pneumonia.

*From the Department of Pathology, Duke University School of Medicine.
Received for publication August 12 1935

These findings caused us to wonder if jaundice such as obtained in this case might not be responsible for low platelet counts and hence an hemorrhagic tendency. We have not found in our reviews of the subject any continuous experimental or clinical observations on blood platelets during the course of an obstructive jaundice, and in view of this fact we have undertaken such a study.

MATERIALS AND METHODS

Rabbits were chosen as experimental animals because of the ease of taking blood samples from the ear and the convenient size of the animal. Adult female animals of three to six pounds weight were used, which were so far as could be ascertained, free from disease. The criteria used for determining the condition of the animals were: (1) condition of fur and general appearance; (2) appearance of viscera at laparotomy; (3) blood picture;* and (4) temperature.†

The diluting fluid used for the counting of platelets and erythrocytes was that of Rees and Ecker.¹⁴

Turek's solution was used as a diluting fluid in counting leucocytes.

Smears were stained with stock Wilson stain.

Blood samples were taken from the marginal vein of the ear at the same time each day to eliminate physiological variations of counts.^{18, 20}

Platelets were counted by the direct method, using a $\frac{1}{100}$ dilution. The maximum error of this method, reckoning from simultaneous counts in practiced hands, is about 10 per cent. The success of the method is directly proportional to the lack of injury to the vessel, to the freeness of the blood flow, and to the rapidity of the dilution. The erythrocytes were counted directly from the same preparation. The leucocytes were counted directly in a $\frac{1}{20}$ dilution of Turek's solution. Hemoglobin calculations were determined by Sahli's method.

Smears were taken by the cover slip method, and stained with Wilson stain.

Drop ether anesthesia was used on all animals and all incisions were treated with collodion dressings. After operation animals were kept as quiet as possible for twenty-four hours and given only a little water. They were then fed routinely.

EXPERIMENT I

After being kept for one week under observation the animals were subjected to laparotomy and the common bile duct doubly ligated as it entered the duodenum. After this they were returned to their cages, where they received the regulation postoperative care. Daily postoperative records of

*The normal ranges of the blood elements were considered to be: Platelets, 250,000-750,000 per c. mm. (13, 14); physiologic variation 30 per cent; R.E.C. 4,500,000-6,250,000 per c. mm. (15); physiologic variation 15 per cent.

Leucocytes: 5,000-14,000 per c. mm. (16), Physiologic variation 40 per cent.

Differential (17):

Polymorphonuclears	43.4% (30-50%)
Lymphocytes	41.8% (30-50%)
Monocytes and transitionals	9.0% (2-16%)
Eosinophiles	2.0% (0.3-5%)
Basophiles	4.3% (2-8%)

†The normal temperature range was considered 38.3-40.8° C. (18).

the blood counts, temperatures, and clinical impressions were made during a period of observation ranging from fourteen to thirty three days. The accompanying graphic representation of the blood findings (Group I) shows the effect of this procedure on the blood of six rabbits which had lived without complications sufficiently long to be considered typical.

Autopsies were done on each animal as soon after death as possible and the tissues fixed in Zenker's solution for microscopic study. A typical autopsy protocol is as follows:

Gross and Microscopic—The animal appeared emaciated and yellow. The sclerae were intensely jaundiced. The peritoneum was shiny and glistening. The peritoneal cavity contained about 15 cc of free fluid. The omentum was adherent to the under surface of the liver. The liver, smaller than normal, was colored a yellowish brown. The portal fields were distinctly outlined. The gallbladder and ducts were extremely dilated, and were filled with small biliary calculi in dark green fluid. Occasionally these structures were found

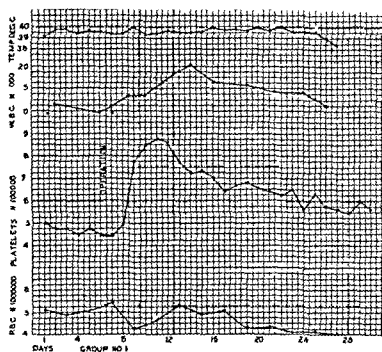


Chart 1

to be filled with white bile. The spleen was greatly enlarged and was soft and spongy to touch. The capsule was very tight. A cross section showed it to be engorged with blood. The malpighian bodies were easily seen.

The lower lobes of the lungs showed some consolidation which appeared to be bronchopneumonia.

Femur bone marrow presented a very great hyperplasia of the red cell forming element and probably also hyperplasia of the white cell progenitors.

The kidneys and heart appeared normal.

Liver The capsule was thickened in some places. The fibrous tissue around the periportal and portal areas was increased more in some places than in others. These areas were filled with proliferating bile ducts, solitary liver cells, occasional small arteries, veins and lymphatics. The area containing the liver cells also showed an occasional bile duct that was 'sprouting' out. Areas of necrosis resembling infarctions of the parenchyma were sometimes found. The liver cells themselves were vacuolated. The nuclei showed no signs of degeneration with the exception of those in the areas of necrosis. The liver cells did not contain large amounts of glycogen; the sinusoids were patent.

Kidneys The capsule was normal. The capillary loops of the glomeruli were engorged. The convoluted tubules were filled with hyaline and fibrous casts, there were places in

which one area showed great enlargement of the lumen with flattening of the epithelium as if by internal pressure. Degenerative processes were evident in some of these cells. This widening of the lumen and flattening of the epithelium was also observed in the loops of Henle and in the collecting ducts, which likewise contained large numbers of hyaline and granular casts. There was some engorgement of the vessels of the kidney.

Spleen: The splenic capsule seemed thinned, and the trabeculae were wide apart. There was an increase in the amount of fibrous tissue that formed the reticulum of the spleen. The sinusoids were filled with red cells, although there was no place in which an actual hemorrhage into the reticulum was seen. The malpighian corpuscles showed a decrease in size and number in proportion to splenic substance.

Lungs: The lungs showed some atelectasis and compensatory emphysema. A bronchopneumonia which appeared to be becoming confluent was evident in the left lower lobe. The bronchi were well filled with polymorphonuclear cells, but no bacteria could be seen.

Bone Marrow: There was a decided hyperplasia of both the red cell forming element and the myeloid forming element, bearing out the gross impressions.

CONTROLS—EXPERIMENT I

In our experimental animals three conditions had been produced that might have affected the rate of production or longevity of the blood platelet: (1) Bile had been backed up into the blood; (2) operative trauma had been produced; and (3) bile had been excluded from the intestine. Since we were interested only in the effect of abnormal concentrations of bile in the blood on the platelet, controls were devised. Our first control, designed to show the effect of operative trauma, is summarized in Chart 2, designated Group 2. Our second control, designed to show the effect of exclusion of the bile from the intestine, is summarized in Chart 3, designated Group 3.

In our first control group, one set of animals was subjected to the same preliminary study as the animals in Group 1 of the experiment, after which a laparotomy was performed. The common duct was picked up, a silk ligature placed around it and loosely tied so as not to obstruct the duct. The wound was repaired, a protective coat of collodion applied to the incision, and the animal returned to its cage. The usual daily record of blood counts, temperatures and clinical impressions was kept until the animal was killed (by a blow on the back of the neck). The postoperative period of observation ranged between fifteen and thirty-eight days. Chart 2 of the blood findings (Group 2) shows the effects of this procedure on the elements of four animals which were considered typical.

A typical autopsy report follows:

Gross and Microscopic.—The animal seemed well nourished; the general appearance was good. The fur was shiny and well kept. Stitches could be found in a well-healed abdominal scar. The peritoneum was shiny and glistening. There were some adhesions of the omentum to the upper part of the duodenum.

The liver, biliary system, kidneys, lungs, spleen, and heart appeared normal.

The femur bone marrow showed a hyperplasia of the red cell forming element, and possibly of the white cell forming element also.

The liver, kidneys, and spleen, microscopically, were normal.

Lungs: There were some areas of atelectasis, and some of emphysema. Occasionally one saw a small area that appeared to be an interstitial reaction, occupying, however, only a very small part of the lung. The bronchi were open and showed no evidence of inflammatory changes in these areas.

Bone Marrow. There was a decided hyperplasia of the red cell forming element, also of the myeloid elements, bearing out the gross impressions

In our second control group (Group 3) the animals were observed for one week during which preliminary studies were made as in our experiment, a laparotomy done, and the common bile duct ligated by double ligatures at its entrance into the duodenum. A bell-shaped glass cannula was fastened in the lumen of

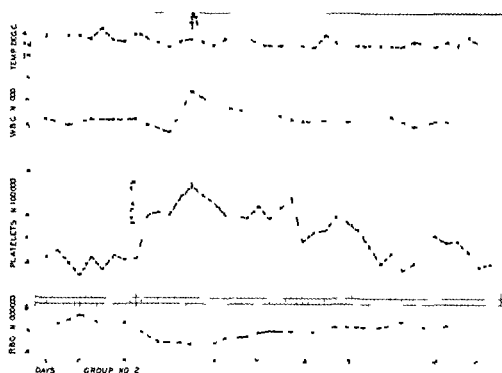


Chart 2

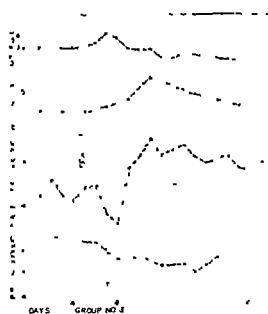


Chart 3

the gallbladder with a purse string. The bile was conveyed from the bladder cannula by means of a small rubber tube, which ran a short way through the subcutaneous tissue along the midline, to the outside of the body. The incision was coated with collodion, and the animals were returned to the cages. They were treated by the usual postoperative technique, care being taken to see that the bile was not ingested. Daily records of blood counts, temperatures, and clinical impressions were kept (Chart 3, Group 3).

A typical autopsy report follows:

Gross and Microscopic.—The animal was extremely emaciated, although the fur was still shiny. The bones protruded through the skin, and there seemed to be no subcutaneous fat. The muscle layers showed an encapsulated layer of leucocytes around the area through which the cannula ran.

The peritoneum was shiny and glistening.

The omentum was wrapped around the cannula, and was plastered up around the gallbladder and the under surface of the liver.

The liver was slightly smaller than normal. It was dark red in color and the portal fields well outlined. The gallbladder was securely wrapped around the cannula. A little fluid and a few small granules were present in the bladder and in the lumen of the cannula.

The spleen was slightly smaller than normal. The cut surface appeared normal, and the malpighian bodies stood out well.

The lungs showed a partial consolidation of the lower lobe of the left lung. The remainder of the left lung and the right lung did not show signs of infection.

The heart and kidneys were grossly normal.

The bone marrow of the femur appeared to show a hyperplasia of the red cell forming elements, and possibly of the white cell forming elements.

In our preliminary study of the animals used in our first experiment and in the above described controls of that experiment, it was shown that a post-operative rise in platelet count always occurred. In order to eliminate entirely this regularly occurring postoperative rise which was thought to be due most likely to trauma of operation, a second experiment was devised as follows:

A fourth set of animals was used, in which the bile duct was occluded by the following procedure without incurring the usual postoperative rise:

Laparotomy was performed and the common duct was surrounded by a loosely tied overhand knot of silver wire. The ends of this were brought through the body wall at the level of the tenth rib, and allowed to remain outside of the body, being first pulled to take up the slack. The abdomen was closed by the usual technic; the ends of the wire were wrapped around sponges and retained in position by means of a tape binder.

The usual postoperative care was observed; blood counts and temperatures were taken occasionally. After a lapse of two or three weeks, observations were made daily for one week before occluding the duct. The occlusion was accomplished by grasping the ends of the wires with straight clamps and pulling evenly on both sides until the knot was felt to be tight.

The blood findings gave no evidence of any constant or otherwise significant variation.

Complete occlusion of the duct was assured by autopsy findings, which were essentially those found in the animals in Group 1.

CONCLUSION

The production of a profound jaundice by the obstruction of the common bile duct produces no quantitative change in the blood platelets in rabbits that are not within the range of error of the method used.

It seems logical, therefore, to believe that the thrombocytopenia in the case observed was not due to the cholemia but coincident with it.

It follows that the explanation of any hemorrhagic tendency associated with jaundice must be sought elsewhere than in a diminution in the number of platelets in the circulating blood due to bile

The author wishes to thank C H Evans, Jr, for aid in starting the problem, C E Isenhour whose aid and suggestions helped to finish it, and Drs W D Forbus, D H Sprunt, and R Baker whose constructive criticisms were invaluable

REFERENCES

- 1 Smith, Greig Abdominal Surgery, Philadelphia, 1891, P Blakiston's Son & Co
- 2 Koechig, I The Calcium Content of the Blood in Pathologic Conditions, J LAB & CLIN MED 9 679, 1924
- 3 Kirk, P L, and King, C G Calcium Distribution in the Blood, J LAB & CLIN MED 11 928, 1926
- 4 Emerson, W C The Distribution of Calcium in Jaundiced and Acoholic Dogs, J LAB & CLIN MED 14 122, 1928
- 5 Walters, W, and Bowler, J P Pre operative Preparation of Patients With Obstructive Jaundice, Surg Gynec Obst 39 200, 1924
- 6 Snell, A M, Greene, C H, and Rowntree, L G A Comparative Study of Certain Tests for Hepatic Function in Experimental Obstructive Jaundice, Arch Int Med 39 273, 1925
- 7 Zimmerman, L M Effect of Parathyroid Hormone on Blood Coagulability, Am J M Sc 174 379, 1927
- 8 Gunther, L, and Greenberg, D M The Diffusible Calcium and the Proteins of Blood Serum in Jaundice, Arch Int Med 45 983, 1930
- 9 Moss, W Experimental Obstructive Jaundice, Its Effect on Fibrinogen and Coagulation of the Blood, Arch Surg 26 1, 1933
- 10 Gram, H C The Result of a New Method for Determining the Fibrin Percentage in Blood and Plasma, Acta med Scandinav 56 107, 1922
- 11 Howell W H Note Upon the Presence of Heparin in Normal and Hemophilic Blood of Man, Am J Physiol 77 689, 1926
- 12 Jones C M, and Minot, G R Infectious Jaundice an Attempt to Establish a Clinical Entity, New England M & S J 189 511 1923
- 13 Casey, A Blood Platelet Counts on Healthy Male Rabbits, J Exper Med 56 841, 1932
- 14 Rees, M, and Ficker, F Effect of Hemorrhage on Complement of the Blood, J Infect Dis 31 361, 1922
- 15 Rosenthal, I Die Sogenannte Pichydenmie der Erythrozyten bei Phenylhydrazin anemie, Folia haemat Arch 10 253 1910
- 16 Graeber, G Ueber die Beziehung von Milz und Knochenmark zu einander ein Beitrage zur Bedeutung der Milz bei Leukaemie, Arch f exper Path u Pharmacol 58 289, 1908
- 17 Scarborough, R A Blood Picture of Normal Laboratory Animals, Yale J Biol & Med 1930
- 18 Meyer, K F The Use of Animals in Routine Diagnostic Work, J LAB & CLIN MED 17 510, 1931
- 19 Hittmar, A Die Blutplattchen, Folia haemat 35 156, 1928
- 20 Bankow, G Sexuelle Hormone in ihrem Einfluss auf die Numerischen Veranderungen die Blutplattchen, Beitz z path Anat u z allg Path 88 113, 1932

PRIMARY CARCINOMA OF THE LUNG*

A REVIEW OF THIRTY CASES

CAROL M. RICE, M.D., MADISON, WIS.

THE statement that primary carcinoma of the lung is on the increase has appeared frequently in the medical literature in the past decade. That statement combined with the fact that three patients with carcinoma of the lung were admitted to the medical service of the Wisconsin General Hospital within a few weeks of each other stimulated interest in this subject and led to the following survey.

At the Wisconsin General Hospital during the years 1925 to 1935, the diagnosis of primary carcinoma of the lung was made in 37 cases. In 30 cases the diagnosis was confirmed; (1) by postmortem examination in 25 cases, (2) by positive biopsy material from bronchoscopic examination in 2, (3) by bronchoscopic examination which revealed an obstructing tumor from which biopsy material could not be taken in two cases, (4) by positive x-ray evidence of a pulmonary neoplasm with concurrent metastases in ilium and femur in one case. Of the other 7 cases the diagnosis could not be confirmed; (1) four died, still having pulmonary signs and symptoms but no postmortem examination was performed and no biopsy had confirmed the impression of carcinoma of the lungs, (2) one died and postmortem examination revealed an adenocarcinoma of the pancreas with metastases to the lung, (3) one in whom the differential diagnoses included pulmonary tuberculosis was sent to a sanatorium for observation and is reported to be greatly improved (both clinically and to x-ray) making carcinoma improbable, (4) one is still alive, but there is no available material for biopsy, and bronchoscopic examination has failed to reveal any lesion although the x-ray findings are very suggestive. Therefore these 7 cases are not included in the survey.

Of the 30 cases reviewed the incidence by years reveals a striking similarity to the reports of others in that there has been a decided increase in the last five years.

YEARS	MALES	FEMALES	TOTAL
1925	0	1	1
1926	0	0	0
1927	0	0	0
1928	1	1	2
1929	2	0	2
1930	3	1	4
1931	2	2	4
1932	2	1	3
1933	3	0	3
1934	4	5	9
1935*	1	1	2
	18	12	30

*To June 1, 1935.

*From the Division of Medicine, University of Wisconsin.
Received for publication, July 6, 1935.

Many theories have been advanced to explain this increase but none has been definitely established

Eighteen of the 30 cases were males and 12 females, giving a sex incidence of 3 to 2. This proportion is unusual since the incidence as recorded by others is usually much greater in the male. Vinson¹ reports a ratio of 4 to 1, Hruby and Sweany² 3 to 1, Olson³ 4.5 to 1. The average age of the males in this series was 55.8 years and that of the females 44.8 years. These figures correspond fairly well with those recorded elsewhere. Hruby and Sweany² found an average age of 49.9 years, Olson³ 53.7 years, and Bonner⁴ concluded that the incidence was greatest in the sixth decade.

The past medical history of the cases in this series is significant in view of the work done by Askanazy (quoted by Hruby²) who found a peculiar regeneration of the bronchial epithelium after influenza and raised the question as to whether this might be a precursor of carcinoma. Although only 15 of the patients gave any history of antecedent pulmonary disease, 13 of these had had influenza and 8 reported a previous pneumonia or pleurisy.

The family histories of these subjects were unusually free from carcinoma. A brother of one patient had died of carcinoma of the pancreas. Otherwise there was no history of malignancy.

The occupations of these patients also appeared to be of little significance. The distribution of indoor and outdoor work was similar to that of the hospital admissions as a whole and the number engaged in occupations with special hazards was no greater than for the unselected cases.

Housewives	8	Not recorded	1
Laborers	4	Lumberjack	1
Farmers	3	Coal miner	1*
Teachers	1	Locomotive fireman	1*
Storekeepers	3	Textile worker	1
Metal workers	3	Electrician	1

*No suggestive history or physical and x-ray evidence of silicosis

Since cough and chest pain are generally considered the two outstanding symptoms of carcinoma of the lung, it is interesting to note that of these 30 cases only 4 offered cough as the chief complaint and only 3, pain in the chest. These symptoms were present at some time in the course of the disease in the majority of the cases, however, only 3 being entirely free from cough, and 5 from pain. An appreciable weight loss was noted in 17 cases.

A survey of the signs reveals a striking incidence of fever. Twenty-five patients had a temperature of 100° or more at some time during the illness. Hemoptysis was reported in 8 cases giving a low percentage as compared to other series of cases (Vinson¹ finding 43 out of 71 cases). The physical signs on examination of the chest varied with the location and character of the primary lesion and the associated conditions such as effusion, abscess, bronchiectasis and atelectasis. In 3 cases the correct diagnosis was suggested primarily by definite signs of bronchial obstruction and in 3 others the signs were directional but not conclusive.

In 9 of the 30 cases bronchoscopic examination was made but revealed a neoplasm in only 4 instances. This low percentage of confirmatory findings is even more striking when one considers that of the 7 cases not included in this

study (of which 6 were very probably carcinoma of the lung) bronchoscopy was negative without exceptions. These results are very disappointing in view of the high percentage of positive findings in some series of cases such as Vinson's.¹ He found positive evidence in 70 of 71 patients examined.

An x-ray of the chest was taken in all but 2 patients. In the latter there had been nothing to suggest any pulmonary pathology. The x-rays revealed the following:

Definite x-ray evidence of carcinoma of the lung	7
Presumptive evidence of carcinoma of the lung	4
Other pulmonary pathology (effusion, atelectasis, etc.)	16
Negative finding	1

An anemia and leucocytosis were apparent in one-third and two-thirds of the cases respectively.

Anemia: Hemoglobin below 60 per cent in 10; average of entire group 63 per cent R.B.C.'s below 4.2 millions in 8; average of entire group 4.2 millions.

Leucocytosis: W.B.C.'s above 10,000 in 20 and above 20,000 in 6; average of entire group 18,000 neutrophils above 85 per cent in 16; average 80 per cent.

A correct antemortem diagnosis was made in 18 of the 30 cases. An analysis of the antemortem diagnosis is as follows:

1. Antemortem diagnosis correct	18
2. Carcinoma of the lung mentioned in the differential	3
3. Diagnosis of pulmonary disease other than carcinoma	5
a. Antemortem diagnosis tuberculosis with effusion, 1	
b. Antemortem diagnosis empyema, 1	
c. Antemortem diagnosis bronchiectasis, 1	
d. Antemortem diagnosis chronic empyema, 1	
e. Antemortem diagnosis hypernephroma with metastases, 1	
4. Nothing antemortem to suggest primary carcinoma of the lung	4
a. Missed abortion with terminal pneumonia, 1	
b. Pernicious vomiting of pregnancy with liver atrophy, 1	
c. Carcinoma of prostate (incorrect), 1	
d. Benign hypertrophy of prostate, 1	

The primary lesion occurred almost equally in the right and left lung and was distributed as follows:

Right		Left	
Lung	3	Lung	3
Primary bronchus	3	Primary bronchus	3
Lower lobe	5	Upper bronchus	6
Lower bronchus	1	Hilum	1
Hilum	1	Pleura	1
	Bilateral fields		2
	Bilateral hilum		1

These figures compare rather closely with those of Olson,³ who found 55 per cent of the squamous cell carcinomas in the left lung and 44 per cent in the right, an equal distribution of the adenocarcinoma, and 56 per cent of the undifferentiated on the left and 43 per cent on the right.

The microscopic report in the 25 postmortem cases showed the following types:

Bronchogenic	11
Adenocarcinoma	8
Alveolar	3
Unusual form	3

Weller's⁵ statement that carcinoma of the lung rarely fails to metastasize is well illustrated in the series of cases under discussion. The following were noted:

Metastases

Liver	6	Pleura	2	Thyroid	1
Lymph nodes	5	Pancreas	2	Pericardium	1
Lung	4	Ribs	2	Heart	1
Adrenals	4	Ovaries	2	Skin	1
Kidneys	3	Brain	2*		

*Brain examined in only 2 cases

As compared with other series there is apparently a very low incidence of metastases to the central nervous system and to bone. This is probably largely explained by the fact that the contents of the cranium were examined in only 2 cases (where the history and physical findings suggested intracranial involvement), and that search for metastases to bone (either by roentgenograms or necropsy) was not made in every instance.

SUMMARY

Thirty cases of primary carcinoma of the lung are reviewed and the significant findings include:

1. Marked increase in its incidence during the past five years
2. Common history of influenza
3. High incidence of fever
4. Infrequency of cough and chest pain as the chief complaint

In other details of clinical course, diagnosis, and necropsy findings this series closely coincides with similar studies in the literature.

REFERENCES

1. Vinson, P. P. Primary Carcinoma of the Bronchus, *Minnesota Med* 15: 15, 1932
2. Hruby, A. J., and Sweeney, H. C. Primary Carcinoma of the Lung, *Arch. Int. Med.* 52: 497, 1933
3. Olson, K. B. Primary Carcinoma of the Lung, *Am. J. Path.* 11: 449, 1935
4. Bonner, L. M. Primary Lung Tumor, *J. A. M. A.* 94: 1044, 1930
5. Weller, C. V. The Pathology of Primary Carcinoma of the Lung, *Arch. Path.* 7: 478, 1929

... ..

... ..

... ..

... ..

DISCUSSION

While we have not followed the *sero* or *thymidine* interval examination of blood serum, we have followed treated and untreated patients over a period of years and have made a practice of requesting second specimens to confirm our original findings in cases where a positive acknowledgment of syphilis was not given. Although in this group there was only a second test on the same individual, it is beyond the bounds of possibility that meteorologic conditions would always be identical when second specimens were taken. If meteorologic changes are really a factor in producing variations in serologic results, such variations must have occurred in many cases of our series, as the time intervals between the original specimen and the later ones were from several days to several months.

The serologic technique employed by us in the modification of the original Wassermann test recommended in Standard Methods of the Division of Laboratories and Research of the New York State Department of Health. We employed two antipens, an alcoholic and a cholesterolized, purchased from the State Laboratory. Our results as compiled in this article are based on the phosporated results which, when they differed from the alcoholic, were higher with only one exception. The agreement between our original alcoholic result and that of the "retake" specimen was just as close as was the agreement between the original cholesterolized and the "retake" results cited in this article.

From the Laboratory of the City Extension Institute.
Received for publication, August 22, 1936.

RESULTS OF OUR STUDY

We have classified Hoverson and Petersen's Wassermann results which consisted of 98 tests in a series of eight cases, as to changes in successive reactions, i. e., a negative result to a doubtful or to a positive or vice versa. For this purpose we have classed all 2+ results, or stronger, as positive, \pm and + as doubtful. These results are tabulated in Table I together with our results on a series of 421 cases of original and second or "retake" specimens.

TABLE I
WASSERMANN RESULTS

SERIES	NO OF CASES	TOTAL TESTS PERFORMED	INSTANCES OF AGREEMENT BETWEEN DIRECTLY SUCCESSIVE TESTS		CHANGE FROM NEGATIVE TO DOUBTFUL OR VICE VERSA		CHANGE FROM NEGATIVE TO POSITIVE OR POSITIVE TO NEGATIVE		CHANGE FROM DOUBTFUL TO POSITIVE OR POSITIVE TO DOUBTFUL	
			NO	%	NO	%	NO	%	NO	%
Series of Hoverson and Petersen	8	103	44	45.83	22	22.9	21	21.9	9	9.37
Authors' series	421	842	384	91.21	16	3.8	2	0.48	19	4.51

In our series the great majority had never had syphilitic treatment. We repeated the Wassermann test on a second blood specimen taken from a few days to several months after the first bleeding. We found our second result exactly the same as the original in 310 instances (245 remained 4+, 20 remained 3+, 10 remained 2+, 11 remained +, 21 remained \pm and 3 remained negative). In 74 instances the change of reaction (as 4+ to 3+, or \pm to +) did not take the test out of the positive or doubtful group in which the original result was classed.

Although Hoverson and Petersen did not compare their corresponding Wassermann and Kahn reactions in their total of 98 tests on 8 individuals where comparison was possible, we have done so in Table II and have further compared them with a series of 1,714 cases previously reported by us.²

TABLE II
COMPARISON OF KAHN AND WASSERMANN REACTIONS

SERIES	CASES	COFFEY'S TESTS PERFORMED	AGREEMENT KAHN AND WASSERMANN		WASSERMANN POSITIVE KAHN NEGATIVE—KAHN POSITIVE WASSERMANN NEGATIVE		WASSERMANN POSITIVE KAHN DOUBTFUL—KAHN POSITIVE WASSERMANN DOUBTFUL		WASSERMANN DOUBTFUL KAHN NEGATIVE—KAHN DOUBTFUL WASSERMANN NEGATIVE	
			NO	%	NO	%	NO	%	NO	%
Series of Hoverson and Petersen	8	98	48	48.98	28	28.57	18	18.37	4	4.08
Authors' series	1714	1714	1589	92.6	28	1.7	44	2.5	53	3.2

Their tests showed an agreement between positive Wassermann and positive Kahn reactions of 25.51 per cent, and a disagreement between negative Wassermann and positive Kahn of 28.57 per cent.

COMMENT

The extreme variations in serologic reactions for syphilis reported by Hoverson and Petersen and their wide divergencies between the results of Kahn and Wassermann tests on identical specimens are contrary to our own and to common experience. That meteorologic changes could account for such inconsistencies is not supported by the results of our study. As noted in Table I our repetitions were in the majority of instances highly consistent with the original tests. If meteorologic variations are such an important factor as Hoverson and Petersen assert, supporting evidence should not be difficult to find in the vast literature which has accumulated on the subject of Wassermann and Kahn reactions.

We believe that Hoverson and Petersen must look elsewhere for a satisfactory explanation of their results.

REFERENCES

1. Hoverson, Emil T., and Petersen, Wm. F.: Meteorologically Conditioned Variability of Serologic Tests in Syphilis, *J. LAB. & CLIN. MED.* 20: 337, 1935.
2. Short, James J., and Kelley, Margaret F.: Incidence of Syphilis in the General Population and a Comparison of the Kahn and Wassermann Tests, *New England J. Med.* 210: 417, 1934.

THE MODIFICATION OF THE HEMATOPOIETIC FUNCTION IN THE RABBIT BY CERTAIN CYCLIC COMPOUNDS*

DAVID ROBERT CLINFANO, H D, PH D, NEW YORK, N Y

THE clinical literature which has accumulated around the subject of agranulocytic angina indicates that certain therapeutic agents whose structures are fundamentally organic ring compounds, play an important rôle in the etiology of the disease. Amidopyrine is the most commonly reported etiologic agent,^{1,2} but cases have been reported where the precipitation of the syndrome has been attributed to dinitrophenol,^{4,5} arisphenamine,⁶ antipyrene,⁷ urea stibamine,⁸ and nivanol⁹ among numerous other substances. The clinical evidence regarding the relationship between amidopyrine and agranulocytosis is sufficiently strong to leave no room for dubiety. Madison and Squier¹ described a patient who had recovered from a previous attack of agranulocytic angina and had shown a normal leucocyte level for a period of ten months. Within three hours of the administration of 5 gr. of amidopyrine, the patient suffered a chill and a recurrence of the acute symptoms. Within twelve hours the granulocytes had disappeared from the circulation. Nor is this an isolated instance, for similar cases have been reported by Benjamin and Biederman,¹⁰ and by Johnson.¹¹ Such evidence as this cannot be disregarded.

Unfortunately, however, the experimental demonstration of this relationship in laboratory animals has not been nearly as convincing. Madison and Squier administered allyl isopropyl barbituric acid together with amidopyrine to eighteen animals in relatively enormous doses. Only one animal in this series showed a diminution in the total number of leucocytes in the peripheral circulation and even this animal did not present a picture which resembled human agranulocytosis, for the granulopenia was accompanied by a marked diminution in the number of erythrocytes. Similarly, Zia and Folkner,⁸ who had previously reported eight cases of agranulocytosis in a series of 72 patients who were being treated with urea stibamine or neostibosan, were unable to show any changes in the blood picture of rabbits that received large quantities of these drugs over a prolonged period of time. These authors, however, did show that the bone marrow of the treated animals became hyperplastic.

In this study the action of a group of cyclic compounds on the hematopoietic tissues was studied with the hope of throwing some light on the nature of the leucotoxic action. These substances included amidopyrine (phenyl dimethyl dimethylamino pyrazolone), antipyrene (phenyl dimethyl pyrazolone), alpha dinitrophenol, catechol and quinone. It was observed that the administration of

*From the Department of Pharmacology, Cornell University Medical College.
Received for publication August 30, 1935.

large doses of amidopyrine or dinitrophenol, ranging from ten to forty times the normal human therapeutic dose, for prolonged periods of time, seemed to produce no change in the peripheral blood picture. An examination of the bone marrow of these animals, however, showed varying degrees of myeloid hyperplasia, areas of degeneration, and a definite suppression of maturation. It was felt that this hyperplasia constituted so serious a modification of the hematopoietic system that the change should manifest itself in the peripheral blood picture. Accordingly, a test was devised to examine the efficiency of the leucogenic system.

Under normal conditions, the leucopoietic apparatus of the rabbit is an organ in an extremely delicate state of equilibrium and the threshold for leucopoietic stimuli is very low. It has been shown¹² that even the intravenous injection of a small quantity of distilled water may be capable of affording such a stimulus. The effects of this stimulation are manifested in the peripheral circulation by a transient leucocytosis which is a true bone marrow reaction, for it is characterized not only by an increase in the total number of granulocytes in the peripheral circulation but also by a marked increase in the number of juvenile cells. This liberation of juvenile cells into the circulation is much more significant than the transient increase in the total number of circulating cells, for the ratio of juvenile cells to mature cells is a much more constant factor than the total number of cells in the peripheral circulation.

The more potent stimulating agents produce a more profound and a more consistent effect. Nucleic acid and sodium nucleinate are examples of such potent stimulating agents and the parenteral administration of either of these substances into a normal rabbit will invariably be followed by a marked increase in the percentage of juvenile cells in the peripheral circulation. Table I is an example of the reaction of a normal rabbit to the intramuscular injection of 5 mg./kilo of sodium nucleinate.

TABLE I

DATE	R.B.C. 10 ⁶	Hb	W.B.C. 10 ³	PERCENTAGE GRANULOCYTES	POLYNUCLEAR COUNT					WEIGHTED MEAN
					I	II	III	IV	V	
9/20	6.2	75	9.2	28	23	33	29	12	3	2.39
9/21	6.2	75	8.1	30	22	33	31	11	3	2.40
9/22	6.4	75	10.4	28	22	31	30	12	5	2.47

Following the intramuscular injection of 5 mg./kg of sodium nucleinate, the following results were obtained:

HOURS AFTER INJECTION	R.B.C. 10 ⁶	Hb	W.B.C. 10 ³	PERCENTAGE GRANULOCYTES	POLYNUCLEAR COUNT					WEIGHTED MEAN
					I	II	III	IV	V	
0	6.4	75	8.2	29	24	32	30	12	2	2.36
1	—	—	8.8	31	30	38	23	8	1	2.12
2	—	—	14.2	37	41	28	21	10	0	2.00
4	—	—	15.1	34	42	28	18	12	0	2.00
8	6.3	74	10.6	34	40	29	20	11	0	2.02
24	—	—	8.6	36	31	39	21	9	0	2.17
48	6.28	75	7.8	32	27	43	23	7	0	2.10
96	6.1	75	8.0	27	25	33	32	9	1	2.28
240	6.2	75	6.8	29	21	32	30	14	3	2.46

Amidopyrine.—A series of eight rabbits was examined and a normal nucleate reaction established for each animal. Seven of the animals received 200 mg./kilo of amidopyrine daily (orally) and the remaining animal was used as a control for the group. Daily observations included a complete blood picture with a record of red cell count, white cell count, hemoglobin content, differential leucocyte count, and polynuclear count¹³ (This experimental procedure was followed for all the substances studied.) Animals were taken from the group at periodic intervals and killed in order to provide bone marrow specimens. The reaction to sodium nucleinate was observed at definite intervals during the course of the experiment. Table I also shows the normal reaction of an experimental animal to the intramuscular injection of 5 mg./kilo of sodium nucleinate prior to the administration of the amidopyrine.

When the normal blood picture had been reestablished this animal received 200 mg./kilo of amidopyrine daily. After the tenth day of this treatment a similar quantity of sodium nucleinate was again administered but this time it failed to elicit the normal reaction as may be observed from an examination of Table II. At this time the control animal responded to the second injection of

TABLE II

DATE	RBC 10 ⁶	Hb	WBC 10 ³	PERCENTAGE GLANULOCYTES	POLYNUCLEAR COUNT					WEIGHTED MEAN
					I	II	III	IV	V	
10/22	58	70	9.2	28	25	5	7	9	4	2.32
10/24	6.05	75	9.6	31	24	11	24	11	5	2.32
10/25	59	75	11.4	27	27	4	25	9	2	2.25

Following the intramuscular injection of 5 mg./kg of sodium nucleinate, the following results were obtained:

DAYS AFTER INJECTION	RBC 10 ⁶	Hb	WBC 10 ³	PERCENTAGE GLANULOCYTES	POLYNUCLEAR COUNT					WEIGHTED MEAN
					I	II	III	IV	V	
0	6.1	74	8.2	27	28	37	27	8	2	2.25
1	—	—	6.8	26	28	36	26	8	4	2.30
2	—	—	7.2	27	26	38	25	9	2	2.23
4	—	—	7.6	28	27	33	27	10	3	2.29
5	—	—	8.6	30	26	34	26	9	3	2.31
24	6.2	71	8.4	29	25	36	24	11	4	2.33
48	6.0	73	7.6	30	29	32	25	12	2	2.26
96	6.05	73	6.9	27	26	35	27	9	3	2.28
240	5.85	76	9.1	29	24	34	28	10	4	2.36

sodium nucleinate in the same manner it responded to the original injection. The animal that had received the amidopyrine showed a perfectly normal blood picture at this time: erythrocytes, leucocytes, and differential leucocyte counts showed no aberration from the normal. An examination of the bone marrow, however, showed a marked degree of myeloid hyperplasia as may be observed by comparing the bone marrow of this animal (Fig. 2) with that of a normal animal (Fig. 1). The general appearance of the animal, as judged by behavior, weight, and feeding, was normal.

The second animal in the group received the intramuscular injection of sodium nucleinate on the fifteenth day, during the course of which time 3,000

mg./kilo of amidopyrine had been administered. No reaction was elicited and an examination of the bone marrow showed an even more marked degree of myeloid hyperplasia together with a definite suppression of maturation. In addition to the myeloid hyperplasia there was a marked increase in the number of megakaryocytes. This animal, in spite of the severe changes in the bone marrow, showed no change in the peripheral blood picture which could be brought out by the usual methods of examination (Fig. 3).

The next animal in this series failed to show a normal nucleinate reaction on the twenty-second day of treatment, but presented no abnormalities of the blood picture. Here the bone marrow showed focal areas of degeneration and necrosis which, on casual examination, seemed to be relegated to the myelogenous

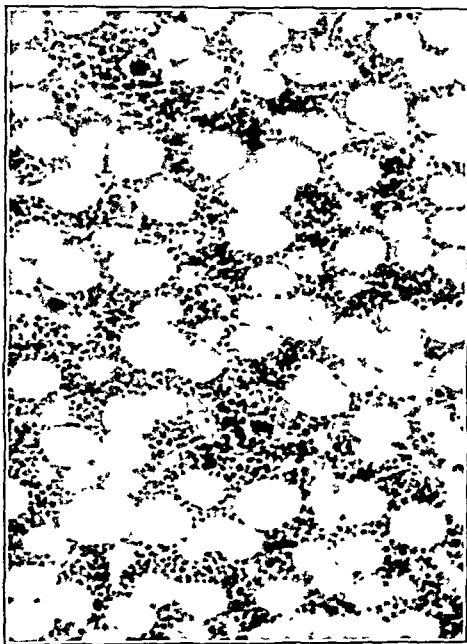


Fig. 1.

Fig. 1.—Femoral bone marrow. Normal rabbit.

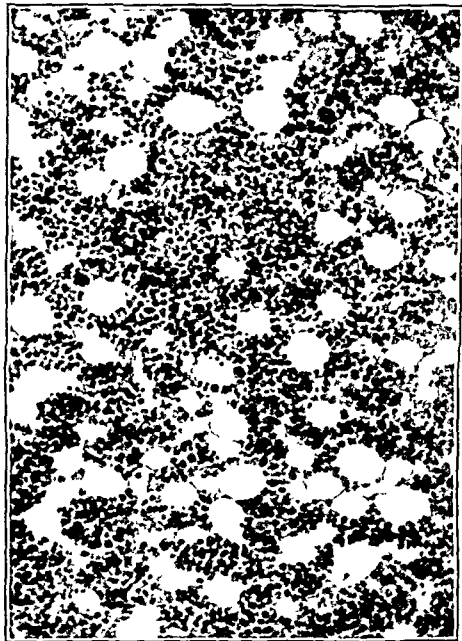


Fig. 2.

Fig. 2.—Femoral bone marrow. Amidopyrine 200 mg. per kilo per day (orally). Tenth day. Marked myeloid hyperplasia.

elements. On closer examination, however, it soon became obvious that there was no specificity about the degeneration and that all the cellular elements were equally affected. The apparent selectivity which was observed may be explained when one realizes the fact that this bone marrow had undergone a massive myeloid hyperplasia and that the predominating cellular elements were cells of the myeloid series.

About the twenty-fifth day the animals of this series began to show an increasing lassitude and anorexia. Only portions of the daily ration were consumed and the animals became listless and inactive. About this time changes began to appear in the peripheral blood picture. These were characterized by

a fall in the erythrocyte level and a fall in the leucocyte level. Red cells fell as low as 3,900,000 and white cells as low as 3,200. The bone marrow at this



Fig 3

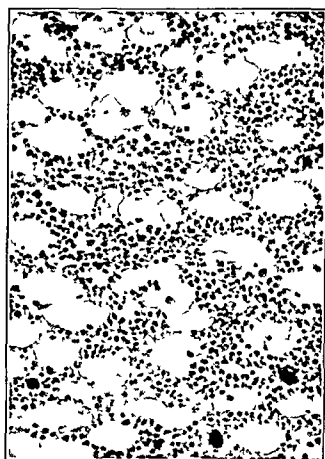


Fig 4

Fig 3—Femoral bone marrow. Amidopyrine 200 mg per kilo per day (orally). Fifteenth day. Marked myeloid hyperplasia with suppression of maturation.

Fig 4—Femoral bone marrow. Amidopyrine 200 mg per kilo per day (orally). Twenty-second day. Areas of necrotic degeneration superimposed on the hyperplastic marrow.

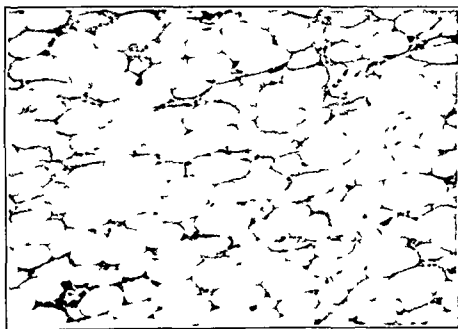


Fig 5—Femoral bone marrow. Amidopyrine 200 mg per kilo per day (orally). Twenty-sixth day. Aplastic marrow.

time was almost completely aplastic (Fig 5). Within two days of the appearance of these symptoms the animal died, death was invariably accompanied by an infection of the upper respiratory tract.

TABLE III
AMIDOPYRINE: 200 MG./KG/DAY (ORALLY)

RABBIT	DAY	R.B.C. 10 ⁶	Hb	W.B.C. 10 ³	PERCENTAGE GRANULOCYTES	NUCLEINATE REACTION
1 (Control)	1	5.8	79	8.6	31	Positive
	3	5.9	79	9.7	33	
	5	5.9	77	10.2	29	
	7	6.1	79	11.2	33	
	9	6.0	80	9.4	31	Positive
	11	6.0	80	8.7	32	
	13	6.2	80	9.2	29	
	15	6.3	79	8.7	29	
	17	6.2	80	11.5	32	Positive
	19	6.1	80	8.2	28	
	21	6.1	80	8.6	27	
	23	6.1	79	10.4	29	
	25	6.1	79	9.8	30	Positive
	27	5.9	80	8.8	30	
	29	5.9	80	7.6	30	
	31	6.0	80	9.8	29	
2	1	6.5	83	11.4	36	Negative
	3	6.3	82	9.8	36	
	5	6.3	82	8.4	36	
	7	6.4	82	10.7	35	
	9	6.3	83	9.4	36	
	10	6.3	83	12.5	36	
3	1	6.8	79	14.5	34	Negative
	3	6.8	79	10.2	34	
	5	6.8	81	12.8	32	
	7	6.4	79	14.2	31	
	9	6.5	80	10.8	34	
	11	6.2	80	11.6	33	
	13	6.4	80	9.8	33	
	15	6.0	80	11.2	33	
4	1	6.25	76	11.2	33	Negative
	3	6.15	74	8.6	33	
	5	6.15	74	7.8	33	
	7	6.2	73	10.4	30	
	9	6.35	74	8.4	32	
	11	6.1	73	10.0	32	
	13	6.15	73	10.8	32	
	15	6.2	73	9.4	31	
	17	6.25	73	9.6	32	
	19	6.0	74	8.5	32	
	21	6.0	72	11.5	31	
	22	5.9	72	10.2	32	
5	1	5.8	79	10.3	29	Negative (died)
	3	5.9	79	8.6	32	
	5	5.8	77	9.8	31	
	7	6.1	79	10.4	29	
	9	6.0	80	8.2	28	
	11	6.35	81	7.4	29	
	13	5.9	74	8.2	27	
	15	6.0	75	7.8	29	
	17	6.0	75	6.8	28	
	19	5.9	72	7.2	28	
	21	5.2	70	6.2	26	
	23	4.8	68	3.6	20	
	25	4.2	65	3.8	19	
	27	3.9	59	3.2	17	

TABLE III—CONT'D

RABBIT	DAY	RBC 10 ⁶	Hb	WBC 10 ³	PERCENTAGE GRANULOCYTES	NUCLEINATE REACTION
6	1	64	77	16.4	24	Negative
	3	64.5	78	14.2	26	
	5	64	77	15.4	25	
	7	62.5	77	16.0	24	
	9	64	76	12.5	27	
	11	64	77	14.0	27	
	13	62	77	15.0	26	
	15	60	74	14.2	25	
	17	62.5	75	10.6	22	
	19	61	74	9.8	21	
	21	59	73	8.5	20	
	23	56.5	70	6.8	18	
	25	52.0	68	7.2	18	
	27	41.0	65	6.4	20	
7	1	67	76	8.2	26	Negative
	3	67.5	76	9.6	26	
	5	68	75	7.3	26	
	7	66	76	8.4	25	
	9	67	76	9.2	24	
	11	67	75	11.3	27	
	13	68	74	9.8	26	
	15	68	75	8.1	25	
	17	64	75	8.0	24	
	19	60	69	7.6	21	
	21	52	68	7.2	21	
	23	50	68	6.8	19	
	25	48	64	4.2	19	
8	1	62	68	5.4	25	(Died on tenth day)
	3	63	68	6.8	25	
	5	62	68	16.4	28	
	7	63.5	68	14.8	30	
	9	63	67	15.2	31	

Table III is an abridged protocol of the results obtained following the administration of amidopyrine. It should be pointed out, however, that the quantity of the drug that was administered each day represents about two thirds of the minimal lethal dose and is equivalent to about 200 gr. of the drug for the normal human adult or approximately forty times the usual therapeutic dose.

Dinitrophenol—The intramuscular administration of 20 mg./kilo/day of dinitrophenol to rabbits produced, save for a number of minor differences, the same changes in the hematopoietic system and in the blood picture as were produced by the administration of amidopyrine. For the sake of brevity, the detailed protocols are omitted. For the first two to five days after the administration of the dinitrophenol, the animals showed a slight but definite leucocytosis which was characterized by an increase in the percentage of juvenile granulocytes in the circulation. This leucocytosis, which was probably an expression of the general metabolic stimulation, returned to the normal level within a period of three to five days. The blood picture was otherwise unaffected for the first twenty five days, after which both the erythrocyte level and the leucocyte level began to fall rather sharply. The nucleinate reaction be

came negative after the twelfth day. When the dinitrophenol had been administered for about twenty-five days, and changes were beginning to be apparent in the blood picture, two of the animals developed sloughing necrotic ulcers in the gluteal region at the site of injection. Both of these animals died three days after the appearance of the ulcer.

Antipyrine.—From a qualitative point of view the action of antipyrine very closely simulated the action of amidopyrine. The fall in the total leucocyte count, however, was not as marked as the fall produced by the administration of either amidopyrine or dinitrophenol. The fall in the erythrocyte count appeared after the sixth day of administration, but was not as precipitous nor did it reach as low a level as the fall in either of the other two series. The nucleinate reaction in this series became negative after the fifteenth day.

Catechol, Quinone.—The action produced by both catechol and quinone was so erratic that the relatively small number of animals used provided no significant results. Doses which were tolerated produced no definite results, while the attempt to increase the dose level only produced symptoms of acute toxicity.

DISCUSSION

The disparity which exists between the clinical evidence as to the etiologic relationship of a group of drugs to agranulocytic angina and the experimental demonstration of this relationship on laboratory animals need not be regarded as directly contradictory. In human agranulocytosis, it is probable that the leucotoxic principle which is involved is not the drug itself, but some oxidation or conjugation product of the drug. In addition to this it is also probable that the leucotoxic action is not a toxic action in the ordinary meaning of the term, but rather a manifestation of an allergotoxic phenomenon with the granulocytes of the peripheral circulation acting as the shock organ. This must be true when one realizes the tremendous number of people who consume large quantities of these drugs without showing any untoward effects.

If the leucotoxic principle is a conjugation or oxidation product of the drug, then it is not surprising to find that experimental animals do not show this specific toxic action, for it is well known that the conjugation of an organic substance may vary from species to species. The classical example of such species variation is to be found in the handling of benzoic acid in the dog and in man. The human being conjugates this substance with glycine, while the dog conjugates it with glucuronic acid. The quantity of glucuronic acid which is available is much greater than the quantity of glycine; therefore, it is not surprising to find that the dog is capable of handling many times the quantity of benzoic acid a man can handle. Variations in the handling of cyclic organic compounds may occur among different individuals of the same species and even in the same individual at different times. This is clearly demonstrated to be the case with cinchopen, for Skorezewski and Sohn¹⁴ have demonstrated that when this substance is administered to a normal individual the greater part of it is converted into oxycinchopen and is excreted in this form. As the length of

time increases during which the drug is being administered, the quantity of oxyechophen which may be recovered diminishes. After a variable period of time the oxyechophen disappears entirely, and the cinchophen is excreted in some other form.

From the experimental findings in this study, it is obvious that the administration of large quantities of amidopyrine, antipyrine, or dinitrophenol is capable of producing deleterious changes in the hematopoietic system. A striking characteristic of these changes is the fact that the bone marrow may undergo serious modification without producing any alteration in the peripheral blood picture which is discernible by the ordinary methods of examination. The application of a test of the functional activity of the leucopoietic system, however, shows that it has been definitely impaired at a time when no obvious changes are present in the blood picture.

The changes which are produced by the experimental administration of these drugs differ markedly from those seen in human agranulocytic angina and more closely approximate those found in cases of phenylhydrazine poisoning. In addition to this fact, the quantity of the drug which is required to produce these changes is far in excess of the dose range encountered in therapeutics. However, it is shown that the administration of these substances is capable of modifying the hematopoietic activity of experimental animals.

SUMMARY

1. A test is described for estimating the efficiency of the leucopoietic function in the rabbit based on the reaction to the parenteral administration of sodium nucleinate.

2. The continued administration of large quantities of amidopyrine, antipyrine, or dinitrophenol impairs the functional activity of the leucopoietic system of the rabbit.

3. This impairment of functional activity is associated with a series of morphologic changes in the bone marrow. These changes are successively (a) myeloid hyperplasia, (b) myeloid hyperplasia with suppression of maturation, (c) focal degeneration and necrosis and (d) aplasia.

REFERENCES

1. Madison, F. W., and Squier, T. I. The Etiology of Primary Granulocytopenia (Agranulocytic Angina), *J. A. M. A.* 102: 755, 1934.
2. Rawls, W. B. Neutropenia Developing During Amidopyrine Medication, *A. J. M. Sc.* 187: 837, 1934.
3. Hoffmann, A. M., Butt, E. M., and Hickey, N. G. Neutropenia Following Amidopyrine, *J. A. M. A.* 102: 1213, 1934.
4. Silver, S. A New Danger in Dinitrophenol Therapy: Agranulocytosis with Fatal Outcome, *J. A. M. A.* 103: 1058, 1934.
5. Davidson, E. M., and Shapiro, M. Neutropenia Following Dinitrophenol, *J. A. M. A.* 103: 480, 1934.
6. Dodd, K., and Wilkenson, S. J. Severe Granulocytic Aplasia of the Bone Marrow, *J. A. M. A.* 90: 663, 1928.
7. Kahlstrom, S. Agranulocytic Angina, *Calif. & West. Med.* 34: 261, 1931.
8. Zia, L. S., and Forkner, C. E. Acute Agranulocytosis of Kalb Azar: Negative Effect of Urea Stibamine and Neostibosan on the Blood of Normal Rabbits, *Proc. Soc. Exper. Biol. & Med.* 32: 536, 1934.
9. Feer, E. Agranulocytic Angina, *Monatsschr. f. Kinderh.* 42: 157, 1929.

10. Benjamin, J. E., and Biederman, J. B.: Agranulocytic Leukopenia. Report of a Case Successfully Treated with X-rays. The Effect of Amidopyrine, J. A. M. A. 103: 1299, 1934.
11. Johnson, W. M.: A Case of Granulopenia Following Amidopyrine with Two Recurrences, J. A. M. A. 103: 1299, 1934.
12. Climenko, D. R.: Studies on the Arneht Count. The Effect of Alterations of the Serum Calcium Level on the Count, Quart. J. Exper. Physiol. 20: 369, 1930.
13. Cooke, W. E., and Ponder, E.: "The Polynuclear Count," London, 1927.
14. Skorzewski, W., and Sohn, I.: Ueber einige in Atophanharnen auftretende charakteristische Reaktionen, Wien. klin. Wchnschr. 24: 1700, 1911.

SODIUM CITRATE—A SPIROCHETICIDE*

ROY S. LEADINGHAM, M.D., ATLANTA, GA.

A TWO per cent solution of sodium citrate is frequently used in the laboratory to prevent coagulation of cover slip preparations of blood and exudates while the specimens are being examined. Its effect upon the bacterial flora of the specimens is not recorded. Commonly used in blood transfusions and internally as an alkalinizing agent, clinically it is not generally considered to possess bactericidal properties.

An observation of the drug's action upon mouth spirochetes may be of interest to those concerned with the prophylaxis and therapy of spirochetal diseases.

A rather thick cover glass specimen from a laryngeal lesion of Vincent's angina was prepared for dark-field examination. It contained many pus cells and a bacterial flora consisting mainly of myriads of Vincent's organisms. The addition of a drop of a 2 per cent sodium citrate solution caused immediate cessation of movement of the spirochetes and apparently eventual dissolution. Repeated examinations gave the same results.

Later, through the courtesy of Dr. Hugh Lokey, whose patient was being examined, the same solution was applied to the surface of the ulcer from which the specimen had been taken. Ten minutes later no Vincent's organisms were found. None were present the next day or the day following, when the patient was dismissed. Should this drug find a place in the local or intravenous treatment of spirochetal infections, it might replace much more expensive and harmful therapy.

* Received for publication, July 20, 1935.

LABORATORY METHODS

INTESTINAL ILLUMINATOR*

A DEVICE FOR DETECTING INTESTINAL LESIONS IN POSTMORTEM SPECIMENS BY REFLECTED AND TRANSMITTED LIGHT

JOSEPH FELSEN, M.D., NEW YORK, N. Y.

THE device pictured is essentially a rectangular box with the bottom and one long side removed and the top replaced by ground or opal glass. Illumination by reflected light is obtained by means of a 60- or 75-watt bulb situated above the glass plate in a built-up portion of the intact long side (A). Illumina-

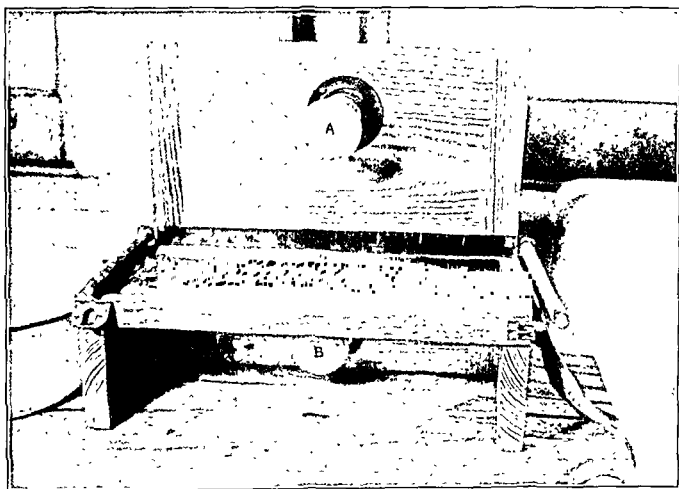


Fig. 1.—Intestinal Illuminator.

tion by transmitted light is obtained by means of a 60 or 75 watt bulb situated below the glass plate (B), thus producing a transparency. The wood is rendered impermeable to moisture by immersion in hot paraffin. Two hinged iron rollers,

*From the Department of Laboratories and Medical Research, Bronx Hospital.
Received for publication, July 2, 1935.

located on either end of the viewing table (i.e., the glass plate), are covered with rubber tubing and serve to hold the opened intestine flat. In routine use as pictured in Fig. 1, the opened washed intestines are brought to the drain board in a basin which may be seen to the left of the intestinal illuminator. The intestines are then fed to the glass plate with mucosal surface uppermost and viewed by reflected light (upper bulb *A* only is lit), the examined portions of the intes-



Fig. 2.



Fig. 3.

Fig. 2.—Focal hemorrhagic necrosis ileum (arrow) above which a Peyer's patch can be faintly discerned.

Fig. 3.—The same specimen as in Fig. 2 viewed as transparency. Note lesions located at ends of arborizations due to vascular necrosis at these points.

tine passing under the second roller and falling into a basin located in the sink. When examination by reflected light has been completed the specimen is reexamined in the same way by transmitted light (lower bulb *B* only is lit). Measurements of lesions or their location may be made by means of a ruler graduated in centimeters attached to the board at the far edge of the viewing table. We

use a strip of old film graduated as above with a distinguishing mark for every 5 cm. After a little practice, examination by both reflected and transmitted light

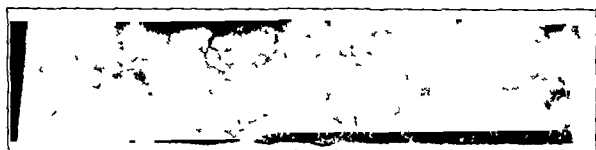


Fig 6

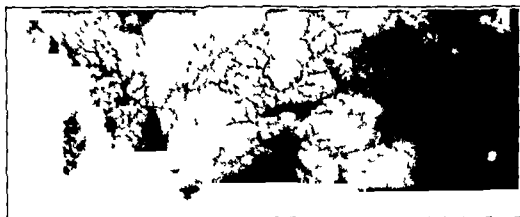


Fig 5



Fig 4

Figs 4 5 and 6—Other transparencies

may be completed in the same operation, the examiner switching off either light as conditions warrant.

This apparatus enables one to detect many intestinal lesions which are missed or not visible in reflected light (see Figs. 2 and 3). A comparative table is given.

Intestinal pictures seen by:

REFLECTED LIGHT	TRANSMITTED LIGHT
Peyer's patches	Blood vessels
Focal lymphoid hyperplasia	Vascular and perivascular lesions
Projecting tumors	Emboli
Ulcers	Focal necroses
Varicosities	Intramural abscesses
Melanosis	Infiltrating tumors
	Small intramural tumors (e.g., leiomyoma)
	Early lesions in Peyer's patches
	Leucemic infiltrations

A PATHOLOGIC LIBRARY OF GROSS SPECIMENS*

NILS P. LARSEN, M.D., HONOLULU, T. II.

DURING the past few years at the Queen's Hospital we have developed a pathologic library which has received considerable comment from visitors as being a plan that should be published. Although there is nothing particularly original in its concept, the use of ordinary laboratory equipment for the development of the pathologic museum may be sufficiently different from the usual to warrant publication.

We have stressed the fact that a general hospital should be the medical center of any community where there is no medical school. With this in mind, for a number of years we collected interesting pathologic specimens in the usual laboratory jar. These are cumbersome and distasteful to handle and do not show the lesion well. They were very difficult to make use of in our weekly clinics, so we began some years ago using the ordinary Petri dish for the mounting of interesting specimens. The method used is as follows:

The specimen is first fixed in Jaure's solution, through which, during the first hour, illuminating gas is passed. It is kept five days in Jaure's solution, then in running water for five hours, and finally in 80 per cent alcohol. From this solution the specimen is cut to fit the Petri dish. The mounting solution is then prepared as follows: In every 1,000 c.c. of distilled water, 75 gm. of gelatin are dissolved. To every 200 c.c. of the gelatin mixture one adds, as the solution is cooling, 25 c.c. of a 40 per cent formalin. As soon as the formalin is added to the gelatin, it must be immediately poured over the specimen. It hardens rapidly. After the specimens are mounted in the above solution and have stood overnight, a thin layer of beeswax and paraffin (1 to 2) is applied. After this is thoroughly dry, a thin layer of plaster of Paris is poured over and levelled. Finally, when this is set and thoroughly

*From the Queen's Hospital.

Received for publication, June 25, 1935.

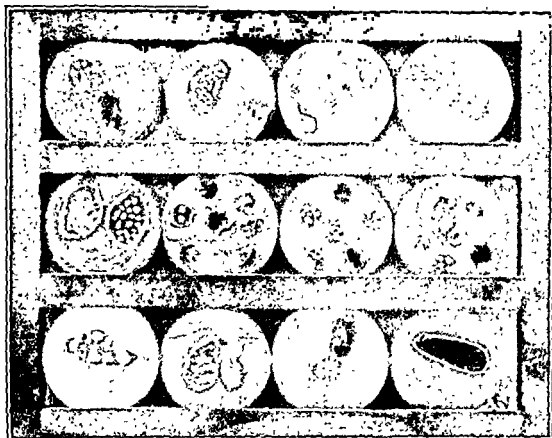


Fig. 1.—Specimen page from pathologic library.



Fig. 2.—Uterus, ovary with corpus luteum, six-week embryo.

dry, another coating of beeswax and paraffin is applied. Recently we have tried battleship cement to seal the plaques. We believe this will make them more permanent. A typewritten caption can be placed in the gelatin as soon as it is hardened.

The house carpenter then built what we call the "pages" for our pathologic library. These are illustrated in Fig. 4. It will be noticed that the plaques can be slid in from the side, so that each "page" contains twelve plaques. Any one can be removed individually and can be handled with impunity. The student or doctor has a more intimate contact with the specimen and can appreciate more fully the gross pathology. Figs. 2 and 3 are of individual plaques. Micro-



Fig. 3.—Cross-section of heart with coronary sclerosis in case of sudden death.

scopic sections are also made and filed with numbers. Hence the microscopist, while he is studying a section, can have a plaque with the gross specimen by the microscope. This, we believe, is of importance, especially to the young pathologist. Fig. 4 shows our whole library as it is arranged in wooden frames above our ice box. It is needless to comment on how much space is saved in such a pathologic museum. A second advantage is, if the pathology or the disease of a certain organ is to be discussed, the whole page showing the variations of pathology in this particular organ can very easily be carried to the clinic room. They are also used in classroom teaching for the nurses.

The total cost of materials and mounting of such a specimen is forty-two cents. If air should get into it, it can very easily be remounted with a minimum of added expense. If air-tight, these plaques have kept very well for several years.

Another use we have made of these is to lend them out to biology teachers of high schools, especially our plaque containing a series of human embryos.

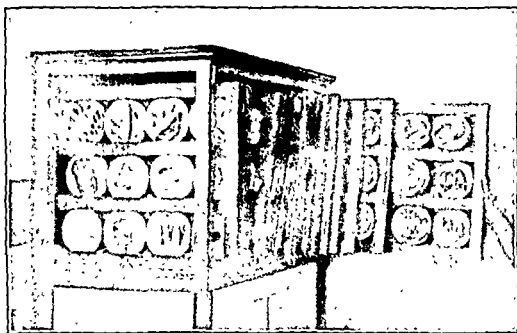


Fig. 4.—Pathologic library complete.

We believe the teaching of biology from human specimens is more interesting than the teaching of biology from bugs and grasshoppers.

A general hospital, if it lives up to its duty as a medical center, should be prepared to help the teachers of the various schools, which can be done by making use of the material available. The hospital laboratory should be prepared to help the schools with all types of laboratory material, and be one of the links in teaching its community what sane, scientific medicine really is.

A RAPID UNIVERSAL BLOOD STAIN*

MAY-GRUENWALD-GIEMSA IN ONE SOLUTION

MAX M. STRUMIA, M.D., BRYN MAWR, PA.

ONE of the commonest and one of the most important daily performances in a clinical laboratory is staining of blood smears. With growing interest in the prognostic and diagnostic value of the leucocytic picture, the necessity of obtaining uniformly well-stained blood smears is obvious. In addition to this, uniformity in staining smears is the first step toward a much needed standardization of blood cell classification. However, in the average clinical laboratory blood smears are inadequately stained and great difficulty experienced in obtaining uniform results. The causes for this common and serious failure are multiple, and it would be beyond the scope of this article to enumerate them. As a rule it is not the inadequacy of the original material but rather the method of employing it which is at fault.

Most of the methods now employed are based on the Romanovsky method which was first introduced in 1891. Of the very numerous modifications which followed, the best is that of Giemsa, although in this country the methods of Wright and of Wilson are more popular, probably because simpler. Giemsa obtained in pure form ethylene azure which is usually known as Azure I. A mixture of equal parts of Azure I and pure methylene blue is called Azure II. In the Giemsa solution this is mixed with Eosin R.A., heated with glycerin and then dissolved in pure methyl alcohol. Pappenheim proposed fixing and staining of the smears with the May-Gruenwald solution (a methylene blue eosin mixture) and then restaining them with diluted Giemsa solution. This method is by far the best that we have at our disposal, correcting the faults of either method, namely the pale color of the erythrocytes and the pale color of neutrophilic granules with the Giemsa alone, and the failure of the May-Gruenwald to stain adequately the azurophilic granulations and the tendency to overstain the nucleus and neutrophilic granulations with resulting loss of important details. Ferrata endorses the same technic with minor changes.¹

The objections to this method are the high cost of the Giemsa solution, which is rather difficult to prepare in the ordinary laboratory, the necessity of using perfectly neutral distilled water, the rather complex technic that allows staining of only a few slides at a time and finally the time required, which is about twenty minutes. The rapid Giemsa method employing a mixture of Giemsa and methyl alcohol or Giemsa acetone in equal parts is a quick and satisfactory staining method for routine work.² Employing a buffered solution for the dilution

*From the Laboratory of the Bryn Mawr Hospital and The Department of Pathology, University of Pennsylvania.

Received for publication, July 15, 1935.

of the Wright stain according to Haden³ has improved the uniformity of results with this solution, without, of course, removing fundamental deficiencies of this stain. These are similar to those mentioned for the May Grunwald, with the added tendency to form precipitates on the smear. The Wright stain has by some workers been substituted for the May Grunwald in using the Pappenheim technic.

It appears therefore very desirable to combine, if possible, the staining properties of the Giemsa method with those of the May Grunwald in one solution, simplifying the preparation of the stain as well as its use, at the same time standardizing the procedure so as to obtain exactly the same result with each smear. This may be accomplished by using a stain prepared as follows: 1.3 gm of finely ground Azure II eosin mixture (Giemsa) are suspended in 80 cc of pure glycerin and allowed to dissolve, shaking occasionally, for two or three days. This mixture is then heated in the water bath at 60° C for two hours, stirring once in a while. The mixture is then cooled and dissolved in the following: methyl alcohol, 290 cc, acetone C P 290 cc. Prepare separately the following solution: methylene blue eosin powder (May Grunwald) 15 cg, methyl alcohol 170 cc, acetone C P 170 cc. It takes several days for the powder to dissolve, shaking occasionally. When entirely dissolved add to the Azure II eosin solution and mix. Occasionally not all of the Azure II-eosin dissolves. It is better to allow it to remain in the bottom of the bottle. It will finally dissolve and if not, it does in no way interfere with the staining property of the solution. The technic for staining is as follows: fresh thin blood smears are covered with the stain and fixed under cover to avoid excessive evaporation for two minutes. Use 1 cc of stain. After two minutes add 1 cc of the alkalinized solution. Mix *thoroughly* and allow to stand for three minutes. Wash under strong running tap water. Air dry and examine. The alkalinized solution is prepared from a stock solution of 1 per cent sodium carbonate in distilled water. Two tenths cubic centimeter of this solution is added to 100 cc of distilled water. This is used for the dilution of the stain on the slide as indicated.

The 1 per cent stock solution of sodium carbonate should be renewed every two months, the diluted solution should be renewed every two or three days.

The preparation of this stain is simple and allows no variations. Its use is rapid and easy, there is freedom from precipitate, and overstaining is not easily done. The stain keeps well, the only thing requiring some attention is the amount of alkali to be employed. In this regard it must be said that dyes from various manufacturers and even different batches of dyes from the same manufacturer vary slightly.

When the solution is first prepared, it is best to allow it to stand for a fortnight or so before determining the amount of alkali necessary, if any.

If the stain is too acid, the color of the slide is bright red, and on microscopic examination the erythrocytes are found to be very bright red, the eosinophilic granules are likewise overstained but the basophilic cytoplasm of lymphocytes and monocytes is practically colorless, the neutrophilic granules are muddy and indistinct, the azurophilic granules are faintly stained, and the nuclei also are very pale. When the solution is too alkaline the smear appears bluish and

microscopic examination shows purplish blue erythrocytes, opaque purplish eosinophilic granules, large overstained neutrophilic granules and azurophilic granules, nuclei very dark with loss of detail and basophilic cytoplasm of lymphocytes and monocytes too deep blue. When the diluent added is of proper reaction, an iridescent scum appears over the stain. This does not give rise to precipitates and is easily washed off.

In a typical experiment stains were prepared with dyes from five manufacturers. Of these three proved satisfactory, two being domestic and one a foreign product. The three satisfactory stains were allowed to stand for fifteen days and then tested. "A" required for diluent 0.1 c.c. of 1 per cent sodium carbonate solution, diluted with 100 c.c. of distilled water. "B" required nothing but distilled water. "C" (the foreign product) required 0.2 c.c. of 1 per cent sodium carbonate solution diluted with 100 c.c. of distilled water. Six months later these same stains were tested again. They had been kept in cork-stoppered brown glass bottles. At this time "A" required 0.2 c.c. of the 1 per cent sodium carbonate solution diluted with 100 c.c. of distilled water. "B" required 0.1 c.c. of 1 per cent sodium carbonate solution diluted with 100 c.c. of distilled water. "C" required 0.3 c.c. of 1 per cent sodium carbonate solution diluted with 100 c.c. of distilled water. It was also found that the time of staining for "C" had increased from three to five minutes.

A year later the dyes were tested again with the result that "A" required 0.6 c.c. of 1 per cent sodium carbonate solution diluted with 100 c.c. of distilled water for staining, "B" required 0.3 c.c. of a 1 per cent sodium carbonate solution diluted with 100 c.c. of distilled water, and "C" required 0.4 c.c. of a 1 per cent sodium carbonate solution diluted with 100 c.c. of distilled water. "C" required seven minutes with the diluted stain, whereas with the other stains, a three-minute period was sufficient. After about two years from the date of preparation, stain "A" required 1 c.c. of 1 per cent sodium carbonate in 100 c.c. of distilled water for the diluent. The stain was allowed to remain for five minutes. Stain "B" required 0.6 c.c. of 1 per cent sodium carbonate in 100 c.c. of distilled water for the diluent, and five minutes for staining. "C" required 1.2 c.c. of 1 per cent sodium carbonate in 100 c.c. of distilled water as diluent, the staining time being seven minutes. After the two-year period the stains begin to deteriorate and the first sign of a permanent loss of staining qualities is the inability to differentiate properly the neutrophilic granules. It may be stated then that except for variations in the concentration of the solution of sodium carbonate used as diluting fluid, the stain can be safely used for a period of one and one-half years from the date of preparation, after which it must be more carefully watched. Solutions two years old or more are not dependable.

This experiment has been repeated and similar results were found on several occasions, namely that as the solution matures it requires slightly larger amounts of alkali and longer time to obtain the same staining. All of the other staining properties remained absolutely unchanged. Tests carried out with numerous other blood stains show that all of them require with aging a change in the diluent. As a matter of fact, the stain here proposed is more stable than any that

we experimented with including the best commercially prepared solutions of the Giemsa stain. If the stain is prepared in small amounts, sufficient for a period of three to six months, hardly any variation in the amount of alkali is required after the first fifteen days. If variations in the amount of alkali are necessary, they are, as one can see, very easily done. As a matter of fact, the possibility of completely controlling individual features of the stain by varying the amount of alkali, and yet reproducing the same general results, is a very important feature of this method. Great latitude is also allowed in the time of staining, although as a rule three minutes of the diluted stain are sufficient, five and even ten minutes will not overstain. In determining the minimum time and the dilution, we have taken mainly into consideration the staining of the nucleus which we consider the essential feature of most cells. The nucleus, in three minutes, will be found stained perfectly, showing the finest details of the chromatin network, as well as coarser structures such as nucleoli, etc. Monocytic nuclei show particularly good differentiation, which makes the cell readily recognizable.

Practically, the titration of a newly prepared batch of stain is carried out as follows: a number of slides of normal blood are covered with 1 c.c. of the stain. After two minutes the diluent is added, 1 c.c. being used, with the following concentration: Slide 1, 1 c.c. of pure distilled water; Slide 2, 1 c.c. of solution prepared by adding 0.1 c.c. of a 1 per cent sodium carbonate solution to 100 c.c. of distilled water; Slide 3, 1 c.c. of a solution similarly prepared, using 0.2 c.c. of the 1 per cent sodium carbonate solution to 100 c.c. of distilled water and so on to a concentration of 0.5. The slide giving the best staining qualities indicates the best concentration for that particular batch of stain. None of the stains showed precipitate on long standing. When the stain is well applied, the slide is grossly of a bright pink purplish color, and at the microscope shows a pleasant pink purplish color of the red cells. Platelets are fully stained, the cytoplasm of lymphocytes and monocytes is pale, transparent blue, allowing a good differentiation from the deeper blue of the Tuerck cells, plasma cells and immature erythroblasts and megaloblasts. The nucleus of the polys and that of the monocytes is lightly, delicately but fully stained showing the structure very distinctly. That of the lymphocyte, of course, is proportionately much heavier stained. The fine azurophilic granules of the monocytes as well as azurophilic granules of the mature lymphocytes and other cells are fully stained. The neutrophilic granules appear very minute and lightly stained in a purplish pink color, allowing a very easy differentiation between the normal granulations as described and the hyperchromic forms occurring in infection (toxic granulations). The abnormal cells, both the immature and the undifferentiated, as well as degenerated ones, are readily stained within the ordinary time. As a rule, bone marrow smears should be stained for a little shorter period, about two thirds of that required for ordinary blood smears.

In addition to staining of blood smears, this stain has been found very satisfactory for the staining of malarial parasites, blood parasites in general, and spirochetes (by staining twelve hours in a Coplin jar, with stain diluted 1:4). We would like once more to emphasize the essential requirements for obtaining

good results as being: first, fresh thin smears; second, exactness in the preparation and dilution of the stain; third, correct amount of alkali; fourth, thorough mixing of stain and diluent.

SUMMARY

A method is given for the preparation and use of a rapid universal stain for blood smears, combining in one solution the staining properties of the May-Gruenwald and of the Giemsa solutions.

NOTE: The stain described in this article is now prepared by the Coleman and Bell Co., and submitted to strict standardization before distribution.

REFERENCES

1. Ferrata, Adolfo: *Le Emopatie* 1: Società Editrice Libreria, Milano, 1933, p. 722. This book contains (pp. 707-725) a most complete list of methods for staining fixed blood smears and a very good literature on the subject.
2. Shilling, Victor: *The Blood Picture*, St. Louis, 1929, The C. V. Mosby Co., p. 31.
3. Haden, Russell L.: *Technic of Blood Examination*, J. LAB. & CLIN. MED. 17: 843, 1932.

A RAPID FLOCCULATION METHOD FOR THE DIAGNOSIS OF SYPHILIS*

FINAL REPORT

F. RYTZ, MINNEAPOLIS, MINN.

THIS flocculation method, as applied to blood and spinal fluid samples submitted to this laboratory by the committee on evaluation of serodiagnostic tests for syphilis in the United States,² has been reported in an earlier publication.¹ A few more or less important modifications are included in this detailed report.

In working out the present rapid flocculation method, mainly two time-consuming factors were to be overcome, the coagulation of the blood and the traditional thirty-minute heating of the serum. It is known that certain proteins, such as peptone in 1 per cent solution, when added to fresh blood, will cause it to coagulate in a few minutes, but when antigen is added to serum derived from such a blood sample, flocculation may take place in normal serum. Heating, however, serves the purpose of coagulation well if the blood sample is placed in a water-bath near, but not above, 60° C., especially if the tube containing the sample is lightly rotated in the bath as motion of the blood facilitates coagulation. The sample is solidly coagulated in from two to three minutes. Clear serum is obtained by centrifugation at high speed for about two minutes. The heating of the blood is no requirement for the test and is only

*From the Clinical Laboratories of the Minneapolis General Hospital.
Received for publication, July 31, 1935.

applied in cases of emergency. In no instance does heating of the blood compensate for necessary heating of the serum in order to activate properly the antibody for flocculation of the antigen.

Heating of the serum for three minutes only is insufficient if the serum, soon after heating, is not treated with ammonium sulphate. It is known that some serums will react without heating, but the flocculate tends to dissipate in a very short time. By this method the flocculate will remain intact for several weeks at room temperature, and flocculation readily takes place, especially in treated cases, although the antibody content is very low. The quantity of half saturated ammonium sulphate applied is one part to three parts of the serum. The purpose of the ammonium sulphate is to precipitate minor quantities of serum protein and, together with it, specific antibody as both are precipitated by the same means.

When applied to the globulin fraction alone of syphilitic serum, redissolved in 0.9 per cent sodium chloride solution, this method constantly gives negative or very weak positive reactions, most probably depending on the accuracy with which the globulin fraction is precipitated. On redissolved total protein from syphilitic serum, strongly positive results are uniformly obtained. On protein from negative serum, negative reactions result.

THE ANTIGEN

An emulsified antigen¹ of high lipid concentration appears necessary for a flocculation reaction in serum prepared as for this test. The antigen is prepared as follows. One hundred grams of Difco beef heart powder are suspended in 500 cc of absolute ethyl alcohol and shaken for two hours. The suspension is then filtered through a heavy grade of filter paper into a liter flask. This is tightly stoppered, the cork being protected with tin foil, and stored in the ice box for twenty four hours during which time a heavy precipitate will form. It is then filtered and the clear extract placed in an evaporating dish and evaporated under an electric fan. A soft brownish wax results. The fanning is continued until the alcohol odor has disappeared, whereupon the wax is dissolved in 175 cc of anesthetic ether and shaken in a tightly stoppered bottle for five minutes. This is stored in a dark place at room temperature until the next day. The ether extract is again shaken for a few minutes and then transferred to centrifuge tubes for centrifugation at high speed for five minutes. The clear supernatant fluid is poured into the evaporating dish and fanned for a few minutes until the contents start to thicken. Then 400 cc of pure acetone are added. A light colored waxy precipitate forms which gradually turns brown. The wax is stirred a few times with an applicator and then left to settle for ten minutes whereupon the acetone is carefully decanted. The residue, in the form of a soft, sticky wax, is fanned until the acetone odor has disappeared. Then the wax is weighed and dissolved in absolute ethyl alcohol in the proportion of 1 gm of wax to 10 cc of alcohol. A brown, glass stoppered bottle is preferred for storing the antigen. The wax is dissolved in the bottle by shaking for a few minutes before it is placed in a water bath at about 55° C for fifteen minutes. It is then stored in a dark place until the next day and filtered through a filter paper of dense texture. Any undissolved wax which may adhere to the walls

of the bottle is rinsed out with ether before the antigen is restored to the bottle. It is important that the antigen does not get in touch with wood, cork, or rubber as this seems to affect the product unfavorably.

Antigen thus prepared is stable for at least two months at room temperature if protected against light and air.

THE EMULSION

The amount of antigen to be used for a given quantity of emulsion is determined by titration. The emulsion is prepared as follows: Into a 50 c.c. centrifuge tube is measured 0.8 c.c. of 0.45 per cent sodium chloride solution to which is added 1 c.c. of 1 per cent cholesterol in absolute ethyl alcohol. The cholesterol is added very slowly down the side of the tube and then lightly rotated, not shaken, for about ten seconds. The amount of antigen as determined by previous titration, as a rule about 0.1 c.c., is added very slowly and then shaken vigorously for one minute before the addition of 2.5 c.c. of 0.9 per cent saline. This is again shaken for one minute when the emulsion is ready for use. If fresh salt solutions are employed, the emulsion will remain stable for about a week at a temperature not above 20° C. It is kept at room temperature and reshaken before use.

ANTIGEN TITRATION

Five emulsion tubes are prepared as described above. Before final addition to each tube of 0.9 per cent saline, various amounts of antigen are measured into the different tubes, beginning with 0.05 c.c., increasing the quantity with 0.05 c.c. for each tube so that the last tube contains 0.25 c.c. of the antigen.

Three rows of five tubes are then placed in a suitable rack. The size of the tube is preferably 10 mm. by 75 mm. Into each tube of the first row is measured 0.15 c.c. of a known negative serum, and into all tubes of the second row is measured 0.15 c.c. of a known weakly positive syphilitic serum. Into each tube of the third row is measured 0.15 c.c. of a known strongly positive serum. The tubes are then placed in a water-bath at 60° C. for three minutes. After removal from the bath 0.05 c.c. of half saturated ammonium sulphate is added to each tube. The rack is shaken for a few seconds in order to redissolve the protein precipitated by the ammonium sulphate. To the first tube in each row is added 0.05 c.c. from emulsion Tube 1, and to the second tube in each row is added the same amount of emulsion from emulsion Tube 2 and so on until all tubes are accounted for. Then 1 c.c. of 0.9 per cent saline is added to all tubes and the contents mixed by shaking. The tubes are shaken in a Kahn shaking device for three minutes, or by hand at the rate of about 275 oscillations per minute. Finally 2.0 c.c. of 0.9 per cent saline are added to each of the tubes. One by one the tubes are slowly inverted three times by placing the index finger over the mouth of the tube. It is important not to shake the flocculate when inverting the tubes. The results are then estimated, preferably over the Fisher lamp for reading Kahns or a similar contrivance. The slanted part of the contents of the tube is examined with a hand lens in establishing the titer. The tube with the weakly positive serum which shows the largest clumps of flocculate

indicates the amount of antigen to be used for the given quantity of emulsion, provided the corresponding tube with known negative serum remains negative, and that the tube with the strongly positive serum registers positiveness markedly above the tube with weakly positive serum. All three factors are taken into consideration in establishing the titer of each new batch of antigen.

Fair results may be obtained by using 0.1 c.c. of antigen for the quantity of emulsion given above without titration, but chances are that some weakly positive reactions will be missed, or negative reactions may be unfavorably affected for convenient reading.

THE TEST

This method requires only one tube for each serum and the entire procedure can be carried out in fifteen minutes.

The serum unit 0.15 c.c., is measured into the respective tubes and the rack placed in a water bath at 60° C. for three minutes. After the rack has been removed from the bath and the serum slightly cooled 0.05 c.c. of half saturated ammonium sulphate is added to each tube. The rack is then shaken for a few seconds in order to redissolve the precipitated protein, whereupon 0.05 c.c. of antigen emulsion is added to each tube. This is mixed by shaking. Then 1 c.c. of 0.9 per cent sodium chloride solution is added to each tube. The rack is shaken vigorously for a few seconds before it is placed in the mechanical shaker and shaken for three minutes at the rate of about 275 oscillations per minute as described under antigen titration. Before reading the results 2 c.c. of 0.9 per cent saline are added to all tubes. Each tube is slowly inverted three times.

A negative test consists of unflocculated emulsion particles. The degree of positiveness is estimated according to the clearness of the contents and the distinctness of the individual clumps of the flocculate.

CONFIRMATORY PROCEDURE

In cases of doubt regarding negative or weakly positive reactions, the tubes are centrifuged at high speed (about 2000 revolutions per minute) for three to four minutes. The fluid is then poured off by quickly inverting the tubes and 1 c.c. of 0.9 per cent salt solution is added to each tube. Before reading, the tube is inverted slowly once without shaking. In a negative reaction the emulsion particles scatter readily. A positive test consists of large, irregular flocculation particles. Even a very weakly positive reaction is more distinct than a strongly positive test by the routine method of reading. Under no circumstances can centrifugation be substituted for shaking, but centrifugation is a valuable means by which to force from the routinely completed flocculation reaction the slightest degree of positiveness. This procedure has no bearing on the specificity of the test. It prevents however, misinterpretation of certain negative reactions.

COMPARATIVE RESULTS ON BLOOD SAMPLES

The relative value of this test in comparison with other flocculation and complement fixation methods, as used in this country, has been published by the committee on evaluation of serodiagnostic tests for syphilis in the United States.²

In performing this test on samples submitted by the committee on evaluation, an effort was made not to report results as "doubtful." The confirmatory method described above is a recent improvement not applied on evaluation samples. In certain groups, "positive" results based on weak 1-plus manifestations would have been clearly negative with the confirmatory method of reading.

In our laboratory, results obtained with this test have been compared with the Kahn and the Wassermann on 5,322 blood samples, of which 350 were from patients undergoing syphilitic treatment. The method here described proved to be 3 per cent more sensitive than the Wassermann, 1 per cent more sensitive, and also 1 per cent more specific, than the Kahn, as performed in this laboratory.

Results based on the 350 treated syphilitics alone, indicated this test to be 11.4 per cent more sensitive than the Kahn, and about 25 per cent more sensitive than the Wassermann reaction.

SPINAL FLUID

The present method as first applied to spinal fluid proved unsatisfactory on a comparatively large number of samples. Research work now attempted on spinal fluid in this laboratory appears at this time promising for a rapid, sensitive flocculation test for syphilis on spinal fluid, the result of which will soon be published.

COMMENT

Physical examination of the flocculate resulting from this reaction does not indicate that the flocculate from a positive test has a greater mass than the antigen emulsion alone from a negative reaction, provided both are properly removed. By microscopic comparison the emulsion appears more bulky than the combined antigen-antibody as though the antibody causes changes in the lipoid crystals rather than adds to the total mass.

The cholesterol particles of the emulsion appear as semitransparent, angular crystals with distinct outlines and a slight tendency to clumping. The antigen of the emulsion is visible, in part, in the form of brownish globules of various size, some of which are probably too small for microscopic recognition among other emulsion particles. After flocculation by action of the antibody, the particles lose their distinct outlines as if the individual particle had shrunk in size. The clumping appears definite.

Various investigators, among them Schmidt and Tuljtschinkaja,³ find that antibodies precipitate mainly with the euglobulin fraction of immune serum. O'Meara,⁴ however, terms his streptococcus antitoxin flocculate "not a precipitate of protein," but the substance is "a specific streptococcal product." Pae-chioni,⁵ through his investigation, concludes that bacteria in the animal body are acted upon by enzymes and that specific antibodies are formed in the process of such bacterial destruction. Witebsky⁶ reported success in separating syphilitic antibody from its antigen combination. He also found it possible to reunite the antibody with the antigen after separation. Agglutinins have likewise been separated from the protein of antityphoid serum.⁷ It has been shown⁸ that rab-

bits inoculated with flocculate from human syphilitic serum develop positive flocculation tests and positive complement fixation tests for syphilis, and that control animals injected with antigen emulsion alone remain negative

In this connection it is of interest that toxin has been flocculated by anti-toxin⁹ Anda and Komiyama¹⁰ regard the ability of the antibody to flocculate an antigen as an indication of the potency of the respective immune serums

Recent investigations, therefore, suggest that serodiagnostic reactions are true antigen antibody combinations

REFERENCES

- 1 Rytz, F A Very Rapid Flocculation Method for the Diagnosis of Syphilis A Preliminary Report, *Am J Clin Path* 5 159, 1935
- 2 Cumming, H S et al The Evaluation of Serodiagnostic Tests for Syphilis in the United States, *J A M A* 104 2083, 1935
- 3 Schmidt, A A, and Tulytschinkaya, Klara Verteilung der Immunkörper nach Serum- und weissenfraktionen, *Ztschr f Immunitätsforsch u exper Therap* 79 311, 1933
- 4 O'Meara, R A Q Streptococcus Toxin Antitoxin Flocculation, *J Path & Bact* 40 371, 1935
- 5 Pacchioni, D Fermenti Ed Anticorpi, *Pathologica* 24 215, 1932
- 6 Witebsky, E Eine Bestatigungsreaktion zur Serodiagnostik der Syphilis, *Ztschr f Immunitätsforsch u exper Therap* 80 323, 1933
- 7 Frankel, Max Method for Separating Antibody From Serum Protein Investigation on a Protein Free Antibody, *Proc Roy Soc, London* 111 165, 1932
- 8 Rytz, F Positive Flocculation Tests in Rabbits Inoculated With Flocculate From Human Syphilitic Serum, *Proc Soc Exper Biol & Med* 32 1501, 1935
- 9 Suzuki, S On the Substance in Diphtheria Serum, Which Participates in Flocculation, *Kiutsato Arch Exper Med* 11 253, 1934
- 10 Anda, K, and Komiyama, T Factors Influencing the Speed of Flocculation, *J Immunol* 28 331, 1935

A RING TYPE STERILIZER FOR USE WITH TISSUE CULTURE FLASKS*

JOSEPH T KING, M D, MINNEAPOLIS, MINN

THE usual method of sterilizing the necks of tissue culture flasks is to heat the neck of the flask with a gas flame Unless very carefully done this procedure causes some breakage of expensive flasks We have also suffered loss of valuable cultures by contamination after opening flasks for feeding when using this method The greatest difficulty has been encountered, however, when it becomes necessary to remove the clot for subculture This is due to the fact that after the clot has been detached from the bottom of the flask for removal it practically fills the neck of the flask as it is being poured out Unless the neck is sterile inside and on the edge, contamination is inevitable

It seemed desirable to have a method which would heat the glass evenly on all sides thereby reducing breakage and also control the degree of heating more accurately

*From the Department of Physiology University of Minnesota
Received for publication July 30 1935

In performing this test on samples submitted by the committee on evaluation, an effort was made not to report results as "doubtful." The confirmatory method described above is a recent improvement not applied on evaluation samples. In certain groups, "positive" results based on weak 1-plus manifestations would have been clearly negative with the confirmatory method of reading.

In our laboratory, results obtained with this test have been compared with the Kahn and the Wassermann on 5,322 blood samples, of which 350 were from patients undergoing syphilitic treatment. The method here described proved to be 3 per cent more sensitive than the Wassermann, 1 per cent more sensitive, and also 1 per cent more specific, than the Kahn, as performed in this laboratory.

Results based on the 350 treated syphilitics alone, indicated this test to be 11.4 per cent more sensitive than the Kahn, and about 25 per cent more sensitive than the Wassermann reaction.

SPINAL FLUID

The present method as first applied to spinal fluid proved unsatisfactory on a comparatively large number of samples. Research work now attempted on spinal fluid in this laboratory appears at this time promising for a rapid, sensitive flocculation test for syphilis on spinal fluid, the result of which will soon be published.

COMMENT

Physical examination of the flocculate resulting from this reaction does not indicate that the flocculate from a positive test has a greater mass than the antigen emulsion alone from a negative reaction, provided both are properly removed. By microscopic comparison the emulsion appears more bulky than the combined antigen-antibody as though the antibody causes changes in the lipid crystals rather than adds to the total mass.

The cholesterol particles of the emulsion appear as semitransparent, angular crystals with distinct outlines and a slight tendency to clumping. The antigen of the emulsion is visible, in part, in the form of brownish globules of various size, some of which are probably too small for microscopic recognition among other emulsion particles. After flocculation by action of the antibody, the particles lose their distinct outlines as if the individual particle had shrunk in size. The clumping appears definite.

Various investigators, among them Schmidt and Tuljtschinkaja,³ find that antibodies precipitate mainly with the euglobulin fraction of immune serum. O'Meara,⁴ however, terms his streptococcus antitoxin flocculate "not a precipitate of protein," but the substance is "a specific streptococcal product." Paechioni,⁵ through his investigation, concludes that bacteria in the animal body are acted upon by enzymes and that specific antibodies are formed in the process of such bacterial destruction. Witebsky⁶ reported success in separating syphilitic antibody from its antigen combination. He also found it possible to reunite the antibody with the antigen after separation. Agglutinins have likewise been separated from the protein of antityphoid serum.⁷ It has been shown⁸ that rab-

The centrifuge tubes are prepared with heparin or glass fragments and corked. An 18 gauge hypodermic needle is driven through the cork. The needle should be $1\frac{1}{2}$ inches long. The hub of the needle is left $\frac{1}{2}$ inch above the top of the cork. The bore is explored with a wire to be certain it is free of cork fragments. Cotton is wrapped around the neck of the flask, the cork, and the exposed part of the shaft of the needle. The cotton is tied tightly with thread. The top part of the flask is now wrapped with a paper towel in such a way that the paper can be removed by slipping it off without unwrapping. The tubes are sterilized by autoclaving.

Syringes are prepared in glass tubes with needles attached and sterilized by dry heat.

After sterilizing the skin with iodine, blood is drawn into the dry syringe. An assistant removes the paper towel from the tube and presents the exposed hub of the needle to the operator who, after removing the needle attached to the syringe, attaches the syringe to the needle projecting through the cork. The flask is so held by the assistant that the hub of the needle is between the tip of the index finger and the thumb. In this manner it is held with firmness sufficient to permit the operator to attach the syringe with a light twisting motion.

The unit is now held in the vertical position and the blood expelled into the flask. This is done by alternately depressing and raising the plunger. In this way air is removed from the flask as the blood enters.

The needle, still attached to the syringe, is now removed from the cork. The cotton is left in place.

If the flask contains heparin, mixing is accomplished by gentle agitation, avoiding throwing the blood into the top of the apparatus. If it contains glass fragments, the blood is allowed to clot. The flasks are then delivered to the laboratory and centrifuged without being opened. (In the case of collecting serum, the clot is broken up by striking the bottom of the flask against the hand before centrifuging.)

From this point the technique is as described in the above mentioned publication.

The technique seems to meet the existing requirements very well. We have had very little trouble with contamination since instituting this method.

A RAPID FLUSHING-WASHING APPARATUS FOR LABORATORY GLASSWARE*

PAUL S. PRICKETT, PH.D., AND ORVILLE W. GARRETT, EVANSVILLE, IND.

A DEVICE used in this laboratory has proved to be so convenient and time saving and also has elicited so much favorable comment from visitors that we decided to publish a description of it in the hope that it would help other laboratories in their dishwashing problems.

The drawing is self-explanatory. The device itself is drawn in solid lines, while the faucet to which it is attached and an A.P.H.A. type dilution bottle, in position to be washed, are shown by broken lines.

The device, as purchased at a hardware store or a beverage supply store for a few cents and called by them a bottle washer,† does not usually have a "press-

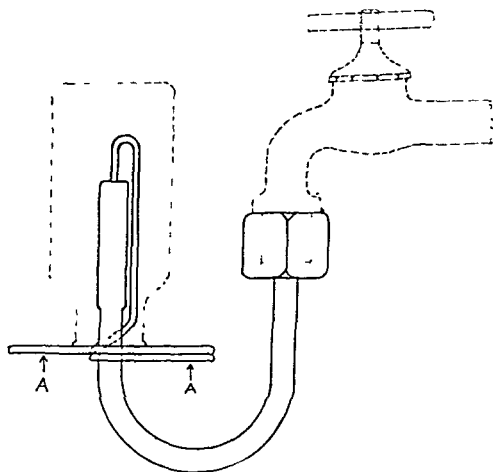


Fig. 1.

ing bar" (A) extending all of the way across, hence we soldered one on and covered it with small rubber tubing to eliminate breakage of glassware.

The water pressure keeps the valve shut when the device is not being used, therefore water is used only for the washing or flushing of glassware, and there is no wastage of water.

We use this apparatus for washing or flushing all test tubes (except serum tubes), bottles, flasks, etc. When dirty pieces of glassware first come to the sink, the dishwasher merely inverts them over the device (see drawing), presses them down and holds them there until the material in them is washed out by the force of the water spray. (Pieces of glassware with narrow necks are washed out faster by removing them from the washer momentarily to allow more rapid drainage.) It is surprising how quickly, conveniently, and thoroughly dirty glassware thus can be washed out.

After the glassware has been thoroughly cleaned by use of brush and soap, the soapy water is then flushed or rinsed out from the glassware's interior, quickly and conveniently, by again inverting it over this device and pressing.

*From the Bacteriological Laboratory, Mead Johnson & Co.
Received for publication, August 20, 1935.

†Freedman Bottle Washer.

PROLONGED PRESERVATION OF THE ANTIGENIC SPECIFICITY OF
SHEEP CORPUSCLES ASSOCIATED WITH THE PRODUCTION OF
A MARKED INCREASE IN RESISTANCE TO
HYPOTONIC SOLUTIONS*

WINIFRED ASHBY, PH D, WASHINGTON, D C

THE tendency of cells to deteriorate is, perhaps, the greatest inconvenience to a laboratory running a complement fixation test for syphilis. Satisfactory preservation of sheep cells for any of the modifications of the Wassermann test is, I believe, generally regarded as a matter of three weeks or less. Kolmer,¹ in the last edition of Kolmer and Boerner, *Laboratory Technic*, claims that cells are satisfactory with formalin for three weeks. With techniques in which the strength of the reaction is judged by the amount of inhibition of hemolysis, rather than by the dilution of serum in which complement fixation takes place, as in the case of the Kolmer Wassermann technique, corpuscles kept for this length of time might not be considered satisfactory. On the other hand, for human corpuscles we have the technique developed by Rous, by means of which blood for transfusion was kept for as long as a month with the addition of isotonic sodium citrate and dextrose solutions. In connection with this technique, Sogen² reported an increase in resistance to hypotonic salt solution.

In 1924, Ashby published data showing that corpuscles in solutions in which they lost their normal cation content, for instance,³ human corpuscles which lost potassium, and dog corpuscles which lost sodium, greatly increased in their resistance to hemolysis by hypotonic solutions. This loss of cation content occurred upon prolonged incubation at 36° C of whole blood in great excess of various isotonic solutions which did not contain the cation normal to the corpuscles. It was shown that the phenomenon of increased resistance to hypotonicity was reversible, if the corpuscles were transferred to isotonic solutions containing the cation normally present. Exceptions, however, were found in certain instances, notably, when an isotonic solution of calcium or barium salts, and, also, if sodium and potassium citrate, had been used as the extracting fluid. It was shown that⁴ the increase in resistance to hemolysis was not due to reduction of intracellular osmotic pressure, as the increased resistance still remained in instances in which it was found by quantitative methods that some other cation had replaced that normally present in equivalent amounts. It was further shown that, in the case of corpuscles whose natural cation content was potassium, the closely related element, rubidium, could⁵ be substituted for potassium with a return of a normal fragility of the corpuscle, while, in the case of the dog and cat corpuscles, which normally have a high sodium content, lithium could be substituted for the sodium with the production of normal fragility. It was

*From the Blackburn Laboratory, Saint Elizabeth's Hospital.
Received for publication July 31 1935

further shown that this phenomenon of increased resistance with the loss of the natural cation could be produced by incubation in appropriate solutions which were above, as well as below, isotonicity.

For these reasons the theory was advanced that the normal cation of the corpuscle bore a chemical relationship, rather than a purely physical one, to the corpuscle hemoglobin stroma complex, and, that the change in resistance to the hypotonic salt solution occurring with the loss of cation was not due to an osmotic pressure effect, but was due to a change in the substance of the corpuscle, resulting in a change in its physical property of solubility in hypotonic salt solution. The probability of the verity of this explanation of the phenomena observed is, I consider, increased by studies, such as those of Van Slyke, Wu, and McLean,⁶ indicating that a considerable portion of the base of corpuscles is present as the cation of a salt of protein.

To produce these results, a period of incubation was necessary which approached that causing deterioration of the corpuscles. For instance, in human corpuscles a three-day incubation period in isotonic sodium chloride solution was necessary to produce the loss of potassium giving the maximum increase in the resistance to hypotonic salt solution; while, with four days' incubation, there was evidence of general deterioration of the corpuscle with hemolysis in the isotonic medium. It was found that, although these results could be obtained with defibrinated blood, they were more easily demonstrated with citrated blood. Citrated blood was used in the major part of this work, and the possible effect of the citrate, per se, was adequately controlled.

Brooks⁷ attempted to repeat this work of Ashby with the blood of some of the animals used by Ashby, but was unsuccessful in his attempt. For a reason which does not seem to me to be adequate, he avoided the use of citration of the blood to be treated, although the effect is more markedly produced with citrated blood, and substituted defibrination. He also omitted to adjust his extraction solutions to pH 7.4 or 7.6, but used them at pH 6.8. His corpuscles deteriorated with hemolysis into the supernatant fluid within two days, and did not show the effect of increased resistance to hypotonic salt solution. The basis of the discrepancy between the results obtained by Brooks and Ashby, respectively, is not, as yet, clear. In addition to the use of the animal corpuscles reported in the work of Ashby, Brooks used sheep corpuscles without any increase in resistance to incubation in isotonic potassium chloride, as had been found in the case of dog corpuscles by Ashby. I have repeated the attempt of Brooks to produce the increased resistance phenomenon by incubation of sheep corpuscles in isotonic solutions of potassium chloride, and have, so far, been equally unsuccessful. But I find that a marked increase in resistance is produced when sheep corpuscles are incubated in an excess of certain isotonic solutions from which both sodium and potassium are absent; incubation in isotonic sucrose, dextrose, or magnesium chloride produces this effect. I conclude, therefore, tentatively, that the substance of the sheep corpuscle is capable of combining with either sodium or potassium to produce the normal state of fragility, and that the preponderance of sodium normally found in the sheep corpuscle is due to the sodium content of the sheep blood serum.

It was reported by Ashby that human corpuscles, which showed very marked increase in resistance to hypotonic salt solution after incubation in isotonic sodium chloride, were still completely sensitive to antihuman hemolysin in the Noguchi modification of the Wassermann test. This marked increase in resistance to hypotonic salt solution upon loss of the natural cation, which would appear to be produced by decrease in the solubility of the hemoglobin, or, perhaps, the hemoglobin stroma complex, suggested to me a state of dormancy of the cell. It seemed probable that, at reduced temperature at which the lipid content of the cell would not be so likely to be lost, it might be possible to keep blood cells for a long time. And, since with human cells, at least, it had been found that their antigenic specificity was not lost with this change in their physical properties, the attempt to preserve sheep cells for the complement fixation test was undertaken.

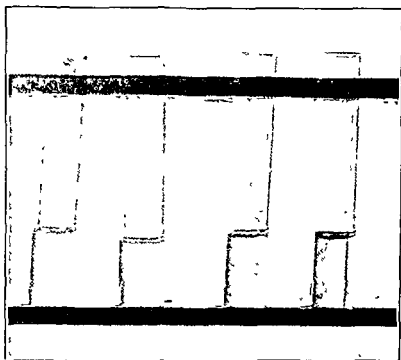


Fig. 1.—Photograph of positive Kolmer-Wassermann test and control run with sheep cells one year and ten days old, illustrating the sharp results obtained with these cells.

Technic and Results.—Attempts were made to preserve sheep cells in hypotonic solutions of sucrose, sodium citrate, dextrose, magnesium chloride, sodium chloride, and mixtures of these with and without the preservatives, formalin and phenol, at room temperatures and at reduced temperatures. Blood was added to the solutions in the proportion of 1 to 10. No adjustment of pH was made. The corpuscles were allowed to sediment and were not resuspended.

The effect of prolonged incubation with formalin was to destroy the antigenic specificity of sheep cells, and, with phenol in preservative amounts, hemolysis took place. With at least certain bacterial growths, specificity was lost, although, in certain instances where a superficial growth of molds had taken place, sensitiveness to hemolysis by antisheep hemolysin was still normal. In view of the fact that preservatives were unsatisfactory, it was necessary to resort to a rigidly sterile technic in taking the blood. The blood was aspirated into a sterile flask to which a bleeding needle was attached with a rubber tubing,

Summary.—A method has been developed for the prolonged preservation of the antigenic specificity of sheep corpuscles.

Results by the Kolmer-Wassermann test comparable to those with fresh cells have been obtained with sheep cells more than a year old.

Prolonged preservation has been accomplished by diluting sheep blood, 1 to 10, with isotonic solutions of sucrose, dextrose, magnesium chloride, and sodium citrate and maintaining the dilutions at 3 to 8° C. Whole blood kept as a control shows signs of deterioration in four weeks.

This development of resistance to autolysis is associated with a great increase in resistance to hypotonic salt solution, and, on the basis of previous work with corpuscles at body temperature, the opinion is expressed that the two phenomena are probably associated with loss of sodium combined with the cell substance.

I wish to acknowledge the technical assistance of Miss Anna Rafferty and of Miss Emera Johnson.

NOTE: Based on work done since this paper was submitted for publication the following suggestions are made for use of the preserved cells in a complement fixation test: The blood should be stored in 3.8 per cent sodium citrate solution. The day before it is to be used the isotonic sodium citrate should be replaced by 0.5 per cent sodium chloride solution. The corpuscles should be resuspended and the mixture returned to the ice box. It will be found that corpuscles so treated wash readily and are free from the undesirable qualities mentioned in the text. With the use of this technic it is unnecessary to bleed the sheep more than four or five times a year. A more complete report will be made on this point.

REFERENCES

1. Kolmer and Boerner: *Approved Laboratory Technic*, New York, 1931, D. Appleton-Century Co., p. 455.
2. Sogen, J.: Die Veränderungen der biologischen und anderer Eigenschaften der lange überlebenden roten Blutkörperchen, *Tohoku, J. Exper. Med.* p. 367, 1920.
3. Ashby, Winifred: A Study of the Mechanism of Change in Resistance of Erythrocytes to Hypotonic Salt Solution. II. Qualitative Study of the Effect of Cations on Human Red Blood Corpuscles, *Am. J. Physiol.* 68: 250, 1924.
4. Idem: Study of the Mechanism of Change in Resistance of Erythrocytes to Hypotonic Salt Solution. III. A Study of the Cause of Effects Produced by Cations on the Resistance of Red Corpuscles Previously Described, *Am. J. Physiol.* 68: 585, 1924.
5. Idem: Study of Mechanism of Change in Resistance of Erythrocytes to Hypotonic Salt Solution. IV. On a Radical Difference in the Effect of Univalent Cations With Reference to Resistance of the Corpuscles of Certain Animals, Which Is Associated With Differences in the Normal Metal Content of the Respective Corpuscles, *Am. J. Physiol.* 68: 611, 1924.
6. Van Slyke, Wu., and McLean: quoted by Peters and Van Slyke: *Quantitative Clinical Chemistry*, Baltimore, 1932, Williams and Wilkins, p. 757.
7. Brooks, S. C.: The Mechanism of Change in Resistance of Erythrocytes to Hypotonic Salt Solutions, *J. Gen. Physiol.* 7: 587, 1925.

A CONVENIENT AND RELIABLE INSTRUMENT FOR ELECTRICAL STIMULATION EXPERIMENTS*

JOHN A. MARRS, M D , BALTIMORE, MD

IN AN attempt made in this laboratory to delimit the motor cortex by electrical stimulation, it became apparent that factors producing great variability in the results of such stimulations existed. This variability in results was found not only in different species, but also in the same species and in the same animal during a single experiment. Even after the factors of temperature and of depth of anesthesia were controlled, some factor of variability remained. This factor could then be ascribed either to the uncontrollable variation in the state of the living tissue itself or to the lack of adequate quantitative measurement of the electric current furnished by the type of instrument used to elicit movement.

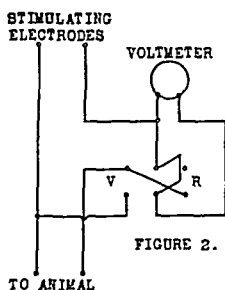
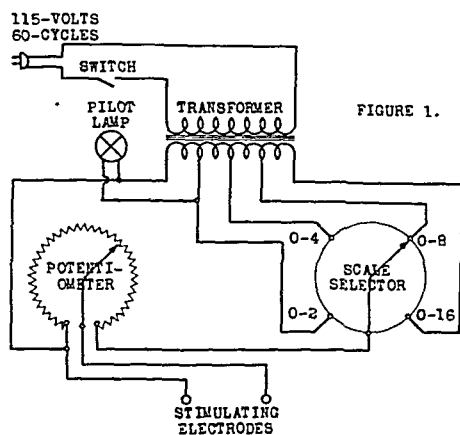
To ascertain whether these variations were inadmissible because they were inherent in the central nervous system or whether the type of stimulating instrument itself contributed an appreciable source of error the characteristics of the inductorium were studied. The inductorium has been used as a source of energy for the greater proportion of all stimulations of the cerebral cortex. This instrument has a nonuniform calibration scale with very coarse adjustment, its frequency setting and stability are poor with cessations of operation due to burning and sticking contacts, the regulation of the instrument is unreliable, for it makes the stimulating voltage a function of the load resistance rather than of the scale setting, the wave shape approaches a triangular form but is inconstant and varies with the magnetic saturation of the iron core and with the vibrator frequency. As these disadvantages are characteristic of this type of instrument a new one was designed and tested upon all of the common laboratory animals.

This instrument was a simple potentiometer circuit designed to be operated directly from a 60 cycle, 115 volt lighting circuit. The wave form of the voltage is practically a sine function so that the maximum value is $\sqrt{2}$ times the effective value (the effective value being that value read by all commercial alternating current meters). There are no contacts to burn or stick, no disturbing noise, and the frequency is constant for indefinite periods of time. Since the stimulating voltage is derived from the voltage drop along a resistance wire, the calibration is a uniform scale and may be made by ordinary linear measurements. Once the total voltage drop of the wire is known this scale can be read directly in volts and subdivided as many times as desired. The current flowing through the wire is adjusted so that its value is very large compared to the stimulating

*From the Department of Anatomy, Johns Hopkins University.
Received for publication July 20 1934.

current, and therefore the regulation of the instrument is excellent. Thus the voltage measured on open circuit is the same as that at any particular instant during stimulation.

This instrument may be constructed in any one of three forms, all of which are in use in this laboratory. The simplest form consists of a resistance-wire of uniform diameter stretched between supports placed at each end of a board. An ammeter and several resistors are placed in series with the wire so that the instrument may be plugged into an outlet of a 115-volt lighting circuit. A hot-wire type of current meter is employed, as by its use the voltage-drop of the



wire may be measured on direct current with a d.c. voltmeter, and the same scale reading of the ammeter on alternating current will give the corresponding *effective* values of the a.c. voltage-drop along the wire.

The second form of this instrument is made on the same principle, the difference being that the straight wire is replaced by two variable rheostats, one 7 ohms, the other 50 ohms, giving fine and coarse adjustment, and the voltage output is measured directly by a sensitive voltmeter. This form requires less space but has the disadvantage that its fineness of calibration is limited by that of the meter, whereas the calibration of the open wire may be spread out indefinitely with slight changes in construction. This form possesses one ad-

vantage not possible with the former in that the resistance of the part under stimulation may be measured quickly at any point desired and the value of the stimulating current calculated

The third form is that which is most suitable for general use both for experimental investigation and for clinical work. A functional schematic diagram of the instrument is shown in Fig 1. The circuit consists of a transformer with a tapped secondary winding which supplies 2, 4, 8, and 16 volts to respective taps on the rotary switch designated "Scale Selector." The voltage of the tap selected is connected across the potentiometer. This fixed voltage impressed across the potentiometer resistance is then varied continuously by movement of the potentiometer contact arm through an arc of 315 degrees. The variable arm of the potentiometer is connected to the output terminals marked "Stimulating Electrodes," so that 0.2, 0.4, 0.8, or 0.16 volts may be obtained for stimulation. Four complete calibration scales are engraved on the panel to designate the intermediate voltage settings of the potentiometer. The full scale values are correct with a line voltage of 115. This gives sufficient accuracy for most work, however, if there is considerable variation in the line voltage of the lighting circuit it is a simple matter to check its value and apply a correction to the calibration scale.

If it is desired to measure the resistance of the part between the stimulating electrodes a Weston Mod 301 Universal A C D C meter may be connected into the circuit as shown in Fig 2. With the switch in position marked *R*, the resistance is determined, in position marked *V*, the voltage at the electrodes is read by the meter. Thus it is possible to obtain quickly and easily the stimulating current by applying Ohm's law $I = E/R$. Measurements of this kind have shown that the resistance of an animal, measured between the pia mater and the skin over the lumbar region, is approximately 2,500 ohms and remains remarkably constant for as much as four hours of continuous stimulation. This resistance does vary according to the type of animal and also between different members of the same species. However, it has been found that the strength of the stimulating current for a species is quite constant regardless of resistance variation. The strength of current required to elicit movement is however, greatly affected by depth of anesthesia. These points will be discussed more fully in another paper.

SUMMARY

A simple alternating current potentiometer is described, this instrument deriving its current from a 115 volt, 60 cycle lighting outlet overcomes all the disadvantages of the inductorium.

A STUDY OF THE RELATIVE SENSITIVITY AND SPECIFICITY OF THE KOLMER-WASSERMANN, KAHN AND EAGLE PRECIPITATION TESTS*

FRED BOERNER, V.M.D., MARGUERITE LUKENS, A.B., AND R. L. GILMAN, M.D.,
PHILADELPHIA, PA.

THE present study was undertaken to compare the Kolmer-Wassermann test, the Kahn precipitation test, and the Eagle precipitation test with respect to sensitivity and specificity, using carefully studied known cases of syphilis for the former, and a normal nonsyphilitic population for the latter. All three technics were carried out as routine tests on the serums used in our study, after first familiarizing ourselves with the Eagle test, which has been but recently added to our routine serologic procedures.

The results obtained by the serologists who participated in the serologic conference recently conducted by the U. S. Public Health Service and the American Society of Clinical Pathologists represent results under comparatively ideal conditions. The tests were carried out by the serologist who devised them and do not necessarily reflect the experience of the average routine diagnostic or hospital laboratory. Our results are therefore described as representative of what may be expected of some of these tests in the hands of the average skilled technician.

MATERIAL AND METHODS

Patients under study or treatment in the syphilis clinic, or syphilitic patients originating in other services comprised the clinical material used as a criterion for sensitivity. Studies for specificity were conducted on a series of selected normal individuals, considered nonsyphilitic on the basis of past history and social status.

Serologic Tests.—The Kolmer-Wassermann test was performed with 0.2 c.c. of serum as the maximum amount as suggested by Boerner and Lukens,¹ the second and third tubes containing 0.1 and 0.05 c.c., respectively. The antigen was the one recommended by Kolmer at the time of this study, and the test as performed has had Kolmer's approval.² The Kahn precipitation test was conducted as described by Kahn,³ with antigen furnished by Dr. Kahn and also with antigen prepared in this laboratory. Results showed both antigens to be equal in sensitivity and specificity. The Eagle test was performed according to Eagle's⁴ technic with antigen furnished by him. As this test has been used by us but a comparatively short time, we ran approximately 1,000 tests before starting the study of comparative tests. The earlier readings were in many instances read or checked by Dr Eagle.

*From the Laboratories and the Syphilis Clinic of the Graduate Hospital, University of Pennsylvania.

Received for publication, July 17, 1935.

Positive readings were given the Kolmer Wassermann of plus one (+1) or stronger. Doubtful readings were those of plus minus (\pm). Kahn readings of average plus one (+1) or stronger were considered positive, while readings of average plus two thirds ($+\frac{2}{3}$) were classed as doubtful and those of plus one third ($+\frac{1}{3}$) as negative. A positive Eagle reading was that in which definite coherent aggregates were formed, persisting on the addition of salt solution, doubtful readings were those in which there was questionable aggregation of the crystals even after recentrifugation as recommended by Eagle.

CLINICAL AND SEROLOGIC RESULTS

A total of 2,730 tests in triplicate were done on as many patients subsequently grouped as follows:

Group A—All tests negative. This group was comprised of proved nonsyphilitic cases, of cases of infection too recently acquired to have demonstrable antibodies by any of the three tests, and of syphilitic cases which had become serologically negative under treatment. This group gives merely the number of cases in which all three tests were in accord in their negative readings and is of no value for purposes of comparison. There were 1,884 patients in this group.

Group B—All tests positive. All cases in this group were considered syphilitic by full clinical confirmation. The patients numbered 483 and when added to the following group gave us a basis for comparing sensitivity (see Table III).

Group C—With one or more tests negative or doubtful (serologic discord). Investigation of the 163 cases in this group revealed in each instance an undoubted syphilitic infection so diagnosed either through previous or subsequent serologic studies or through clinical evidence. The majority of these cases came from our syphilis clinic and represented in the large majority cases under treatment. The data in connection with Group C are shown in Table II.

Group D—Certainly nonsyphilitic. This group included only individuals who were carefully and extensively studied or were intelligent volunteers familiar with the object of this study and who had every reason to believe themselves free from infection. This group constituted 200 individuals and all gave negative results to the three tests performed. This class is interesting only on confirming the specificity of all three tests.

COMMENTS

The data indicating specificity of the three tests are tabulated in Table I, the cases with serologic disagreement, arranged as to clinical types of syphilis, are summarized in Table II. It will be noted that few if any early untreated syphilis cases fall in this category. It has been shown many times previously that the three tests individually are entirely adequate in detecting early infection. It is in latent and late cases where positive serology is most important in diagnosis that we most frequently encounter serologic conflicts. Of growing importance is the serologic progress of the patient under treatment. The progress in any case will be influenced to a great extent by the sensitivity of the test or combination of tests used as a criterion.

TABLE I
A COMPARISON OF THE SPECIFICITY AND SENSITIVITY OF THE WASSERMANN (KOLMER), KAHN AND EAGLE TESTS

	WASSERMANN TEST (KOLMER)						KAHN TEST			EAGLE TEST		
	0.2 C.C. OF SERUM			0.1 C.C. OF SERUM			POS.	DOUBT.	NEG.	POS.	DOUBT.	NEG.
	POS.	DOUBT.	NEG.	POS.	DOUBT.	NEG.						
Certainly syphilis (Groups B and C)	546	21	79	516	17	113	531	34	81	621	9	23
Certainly nonsyphilitic (Group D)	0	0	200	0	0	200	0	0	200	0	1	199

TABLE II
163 CASES OF SYPHILIS IN WHICH THE SEROLOGIC TESTS WERE NOT IN AGREEMENT (GROUP C)

TEST	CONGENITAL			EARLY (TREATED)			LATE (UNTREATED)			LATE (TREATED)			TOTAL		
	POS.	DOUBT.	NEG.	POS.	DOUBT.	NEG.	POS.	DOUBT.	NEG.	POS.	DOUBT.	NEG.	POS.	DOUBT.	NEG.
Wassermann 0.1 c.c.	2	3	14	0	1	11	5	2	4	26	11	84	33	17	113
Wassermann 0.2 c.c.	6	2	11	1	3	8	8	0	3	48	16	57	63	21	79
Kahn test	5	4	10	4	5	3	1	3	7	38	22	61	48	34	81
Eagle test	16	0	3	10	0	2	9	1	1	103	1	17	138	9	23

TABLE III

A COMPARISON OF THE SENSITIVITY OF THE WASSELMANN (KOLMER), KAHN AND EAGLE TESTS WHEN USED ALONE AND IN COMBINATIONS (RESULTS OBTAINED WITH 646 SYPHILITIC SERUMS FROM PATIENTS INCLUDED IN GROUPS B AND C)

	POSITIVE		DOUBTFUL		NEGATIVE	
	NUMBER	PER CENT	NUMBER	PER CENT	NUMBER	PER CENT
Wassermann 0.2 cc-Eagle-Kahn	634	98.1	12	1.8	0	0
Wassermann 0.1 cc-Eagle-Kahn	631	97.6	9	1.3	6	0.9
Wassermann 0.2 cc-Eagle	631	97.6	5	0.7	10	1.5
Wassermann 0.1 cc-Eagle	627	97.0	3	0.4	16	2.4
Eagle-Kahn	627	96.4	8	1.2	15	2.3
Eagle	621	96.1	2	0.3	2	0.5
Wassermann 0.2 cc-Kahn	587	90.2	28	4.3	35	5.4
Wassermann 0.1 cc-Kahn	569	88.0	28	4.8	52	8.0
Wassermann 0.2 cc	546	84.5	21	3.2	79	12.2
Kahn	531	82.1	34	5.2	81	12.5
Wassermann 0.1 cc	516	79.8	17	2.6	113	17.4

In Table III may be seen the relative sensitivity of the three tests under study, both alone and in combination, in the diagnosis of syphilis or the detection of antibodies in late and treated cases. As a single test the Eagle (96.1 per cent pos) exceeded the Kolmer Wassermann 0.2 cc (84.5 per cent pos) and the Kahn (82.1 per cent pos). The Kolmer Wassermann, using 0.2 cc of serum as the maximum dose as recommended by Boerner and Lukens¹ and adopted by Kolmer,² gave 23 per cent more positives than the Kahn and 47 per cent positives than the same test using a maximum of 0.1 cc as originally recommended by Kolmer. The Kahn test gave 23 per cent more positives than the Kolmer Wassermann with 0.1 cc as the maximum dose. It is to be noted that the Eagle test gave but 2 doubtfuls whereas the Kahn gave 34, the Wassermann with 0.2 cc, 21 and with 0.1 cc, 17. These tests should be given credit for not entirely missing these cases. It appears to us that the classification of these reactions as doubtful is due to the conservative methods of reading these tests rather than to the tests themselves. For this reason it may be fairest to the tests to compare the number of negatives which represents the number entirely missed. The Eagle test (3.5 per cent neg) gave less negatives than the Wassermann 0.2 cc (12.2 per cent neg) or the Kahn (12.5 per cent neg). The Wassermann 0.2 cc and the Kahn are about equal both giving about 5 per cent less negatives than the Wassermann 0.1 cc. The relative sensitivity of the three tests remain about the same whether judged by the number of positives or negatives.

As might be expected, a combination of all three tests gave the greatest number of positive reactions. The showing of the Kolmer Wassermann 0.2 cc and Eagle tests combined (97 per cent pos) greatly exceeded the usually duplicated tests, Kolmer Wassermann 0.1 cc and Kahn tests (88 per cent pos), and gave 12 per cent more positives than the Eagle and Kahn combination.

The relative specificity of the three tests is shown in Table I. The Wassermann and Kahn with 200 certainly nonsyphilitic serums gave 100 per cent negative results, while the Eagle gave one doubtful and 99.5 per cent negative. Although this series is small it gives us a fair idea as to the chances of false positive reactions. All three tests failed to give a false positive reaction. The false doubtful reaction obtained with the Eagle test was repeated with the same

result thus eliminating the possibility of technical error. If we consider one false doubtful in 200, a serious offense then, it merely means that our interpretation of the Eagle test should be corrected to the extent that all reactions now read as doubtful should be classed as negative. This change would make it equal to the other tests as regards specificity without appreciable altering its high degree of sensitivity.

CONCLUSIONS

1. In a series of tests with 646 syphilitic serums employing the Kolmer-Wassermann, Kahn and Eagle tests, the Eagle test greatly exceeded the Wassermann or Kahn tests in the number of positive reactions.

2. The Eagle test alone proved more sensitive than the Wassermann and Kahn combined.

3. The Wassermann using 0.2 c.c. as the maximum dose was more sensitive than when 0.1 c.c. was used and about equal to the Kahn test.

4. The best combination of two tests was the Wassermann using 0.2 c.c. of serum and the Eagle test.

5. The Wassermann and Kahn tests failed to give a single false positive reaction with 200 serums from certainly nonsyphilitic individuals. The Eagle test gave one doubtful reaction.

REFERENCES

1. Boerner, F., and Lukens, M.: Some Modifications of the Kolmer-Wassermann Test, *Am. J. Syph. & Neurol.* 19: 489, 1935.
2. Personal communication.
3. Kahn, R. L.: *Serum Diagnosis of Syphilis by Precipitation*, Baltimore, 1925, Williams and Wilkins Company.
4. Eagle, H.: Studies in the Serology of Syphilis. VIII. A New Flocculation Test for the Serum Diagnosis of Syphilis, *J. LAB. & CLIN. MED.* 17: 737, 1932.
5. Kolmer, J. A.: *Changes in the Technic of the Kolmer-Wassermann Test*, *Am. J. Syph. & Neurol.* 19: 481, 1935.

THE ESTIMATION OF THE PHOSPHOLIPID CONTENT OF THE WHITE BLOOD CELLS*

LIDON M. BOYD, KINGSTON, CANADA

IN A NUMBER of recent communications^{1, 2} variations in the lipid composition of the white blood cells have been reported in health and disease. The results of this series of investigations have served to emphasize two facts of prime importance: (a) leucocytoses which may be indistinguishable by microscopic examination of the blood may yet be entirely different by chemical analysis of the white blood cells. A search into the cause of this difference led to the discovery and establishment of the second fact that (b) active leucocytes in patients who recovered normally from fever, infection, surgical operations, or parturition possessed a lipid composition markedly different from that of inactive leucocytes in patients who succumbed to infection or in whom convalescence was prolonged.

Although each of the lipids shown to be present in the white blood cells was found to vary with activity of the blood leucocytes, the changes in one lipid stood out as most consistent and most extensive. This lipid was phospholipid and its relation to activity was found to be analogous to that reported by Bloom and his associates³ in studies of the physiology of glands and muscles and by Boyd in the ovaries⁴ and the fetus and placenta⁵ of rabbits. Briefly, it was shown that active leucocytes contain large percentages of phospholipid while inactive leucocytes contain much smaller amounts.

Having established this fact, the author has made use of phospholipid determinations on the white blood cells in arriving at an opinion as to the chances for recovery of patients referred to hospital with severe infections, marked febrile reactions, postoperative fevers, etc. From observations made to date, it would appear that in the absence of any potent toxin (as in tetanus) the following prognoses may be attached to phospholipid percentages in the blood leucocytes of *infected cases*:

500 mg per cent or less	very poor
500-900 mg per cent	prior to questionable
900-1300 mg per cent	fair to good
1300-1600 mg per cent	very good
Over 1600 mg per cent	excellent

These delineations may require slight shifts in the light of further and more extensive investigations but they are offered from present experience with this method as guides for those who may desire to use it. The prognosis as so estimated is valid in those cases where recovery depends only upon the resistance of the body to infection. When some powerful toxin is present, the resistance

*From the Department of Pharmacology, Queen's University and the Richardson Laboratories, Kingston General Hospital.

Received for publication July 29, 1935

to infection as calculated from the leucocytic phospholipid may be high but the patient succumbs to the effects of the toxin. For example, a case of incomplete abortion complicated by tetanus infection was found to have a leucocyte phospholipid of 1,400 mg. per cent but this patient died from tetanus intoxication a few days later.

Having found the determination of phospholipid in the white blood cells to be of prognostic value, it was considered advisable to report improvements in the technic of isolating the white cells from blood, to gather together various modifications adopted in the estimation of phospholipid, in brief to outline the method now used by the author along with data on the experimental variation or error in technic which may be expected with this procedure.

The amount of blood needed depends upon the white cell count. When this is under 10,000 cells per c.mm., 25 c.c. of blood are required. As the white count increases, progressively less blood is needed; at 20,000, about 10 to 15 c.c., etc. Since the effect of ingested food upon the phospholipid content of the white blood cells has not as yet been determined, it is advisable to obtain a fasting specimen. It is probable that meals do not have a pronounced effect since the author has often been unable to insist upon fasting blood and yet the prognosis determined from leucocyte analysis has been unappreciably affected.

Blood is collected in oxalate or citrate bottles and centrifuged at full speed for one-half to one hour. Thorough centrifuging aids in the cleaner separation of the leucocytes from the plasma above and the red blood cells below. About 2 feet of rubber tubing are attached to a 25 c.c. pipette and the free end of the rubber tubing held between the teeth. The plasma is then drawn off to within a quarter of an inch or so of the white cell layer, a little air drawn into the pipette, the rubber tube tightly clamped between the teeth and the plasma in the pipette discarded. Successive repetitions of this procedure remove most of the plasma and the last trace is absorbed upon a strip of filter paper carefully edged down the side of the centrifuge tube.

A pair of wide tissue forceps are then introduced into the centrifuge tube, and it is occasionally possible to remove all of the white cells in one adherent piece. The forceps should not be closed too tightly over the leucocytes. The removed white cells are then placed on a watch glass, which has been previously cleaned in sulphuric acid-potassium dichromate cleaning mixture and thoroughly washed, dried, and weighed. As many as possible of the red cells and plasma are removed from the edges of the white cells on the watch glass with strips of filter paper. In this manner, what the author has termed the "leucocyte fraction" is isolated and weighed. Invariably it contains traces of red cells and plasma as impurities but analysis of this fraction approximates very closely the analysis of pure leucocytes⁵ and use of this fraction has the distinct advantage of requiring much less blood than is needed if the cells are washed. Also it is possible that washing might affect the percentage of lipids present in the white blood cells.

When the leucocytes cannot be removed in toto as one piece, a slightly different procedure requiring some practice is resorted to. The tips of the tissue forceps are opened and dipped slightly below the leucocyte layer, beginning at one edge. They are then drawn up slowly, gradually bringing the ends of the

forceps almost but not quite together. A small globule of the white cells will thus be caught between the ends of the tissue forceps, and it is placed upon the weighed watch glass. This procedure is repeated until all of the leucocyte layer has been removed. In the majority of cases, this is the manner in which the white cells will have to be separated from blood.

After weighing (usually 0.15 to 0.2 gm obtained), the leucocytes are covered with 2 to 5 cc of sand (alcohol extracted, ignited sea sand) which is mixed with the cells to form a semidry mass. This prevents the white cells from clumping when they are next transferred quantitatively with a cleaned metal spatula to a 125 cc Erlenmeyer flask. Roughly 50 cc of alcohol ether (3:1, both redistilled and the ether peroxide free after Bloor¹⁰) are added and the flask immediately and thoroughly shaken. Small portions of alcohol ether are placed on the watch glass, the residue of adherent cells completely worked off with the spatula and these washings added to the Erlenmeyer flask. The contents of the flask are then brought to boiling for a few minutes on a steam bath, cooled, and filtered. If more than one lipid is to be estimated, the filtrate may be made up to volume and aliquots taken, but for phospholipid alone (or phospholipid and free cholesterol, the latter determined on the acetone mother liquor as below) the filtrate need not be made up to volume. It is advisable to use filter paper which has been extracted with alcohol in continuous extractors for twelve hours to remove any possible lipid material. The residue of sand and precipitated leucocyte protein is washed several times with small portions of alcohol ether, collecting the washings with the original filtrate. All glassware used must be previously cleaned with chromic acid, thoroughly rinsed with tap water and distilled water and dried by flaming.

The resulting alcohol ether extract contains all of the lipids present in the leucocytes and some nonlipoidal material soluble in alcohol ether. These impurities are removed by evaporating off the alcohol ether and redissolving the lipids of the residue in petroleum ether which is a much more selective solvent for lipids than alcohol ether. The procedure for determining phospholipid is based upon Bloor's method of 1929¹¹ with modifications at various times introduced by Boyd^{1, 12, 13}.

The alcohol ether extract contained in a 100 cc tall beaker is evaporated almost to dryness on an electric hot plate using a glass rod with an unglazed end to prevent bumping. The beaker and contents are then transferred to the steam bath where all the solvent is evaporated off at a temperature of about 80° C. Then 2 to 3 cc of petroleum ether (redistilled after standing over concentrated sulphuric acid at least twenty-four hours) are added brought to a gentle boil on the steam bath and the resulting solution of lipids poured off into a cleaned graduated 15 cc centrifuge tube. This is repeated 4 times with small portions of petroleum ether to make a final volume of 10 to 12 cc in the centrifuge tube. Ordinarily none of the thin layer on the bottom of the beaker will dislodge but if any flakes or visible material are carried over into the petroleum ether solution in the centrifuge tube, the latter must be centrifuged and the clear supernatant solution transferred quantitatively to another tube.

A cleaned, dry glass rod of $\frac{3}{16}$ inch diameter is placed in the centrifuge tube and the petroleum ether evaporated to a volume of somewhat less than 1

c.c. by immersing the lower half inch of the tube in about a quarter of an inch of boiling water contained in a beaker. The glass rod effectively prevents bumping by forming a small pocket of volatilized solvent in the conical bottom of the centrifuge tube. The glass rod is then removed and washed into the tube with a very small amount of petroleum ether. The petroleum ether solution of the lipids is then made up to 1 c.c.

The phospholipids are precipitated from this solution by adding 7 c.c. of dried (calcium chloride) redistilled acetone followed by 0.1 c.c. of 30 per cent magnesium chloride in 95 per cent alcohol, mixing and allowing one-half an hour for complete precipitation. They are then centrifuged out and the acetone mother liquor poured off, being cautious not to dislodge any particles of the precipitate. The precipitate is washed once or twice with 2 c.c. of dried acetone, centrifuging and decanting each time. The tube is then inverted to drain, dried with a gentle stream of air and the precipitate dissolved in 10 c.c. of ether (redistilled and peroxide-free) which is saturated with distilled water (a layer of distilled water is kept in the bottom of this ether reagent bottle). The precipitate is dislodged with a finely pointed glass rod, the whole stirred several times during an interval of fifteen minutes or more allowed for solution of the phospholipids. A whitish material is left undissolved which consists mostly of magnesium chloride together with some diaminophospholipid¹³ and cerebroside, if the latter be present. This material is centrifuged out and the supernatant solution of the phospholipids drawn over by suction through a glass tube into a 125 c.c. glass stoppered Erlenmeyer flask (oxidation flask). The glass tube is rinsed several times with ether, the rinsings being caught in the oxidation flask. The ether is then evaporated off at a low temperature on the steam bath, the last traces of ether vapor removed with a gentle stream of air and the phospholipids are left as a thin scum on the bottom of the oxidation flask. Once all the ether has been evaporated off, the flasks should not be left on the steam bath, else the phospholipids may be partially oxidized and low readings obtained.

The quantity of phospholipid thus isolated is determined by oxidation, 1 mg. of phospholipid requiring 3 c.c. of 0.1 N potassium dichromate to completely oxidize it.¹ Oxidation is carried out by adding to the oxidation flask exactly 5 c.c. of 2 per cent silver chromate in concentrated sulphuric acid (prepared after Bloor¹¹) followed by exactly 3 c.c. of 1 N potassium dichromate. The flask is shaken, loosely stoppered and placed on a cast iron plate, $\frac{1}{2}$ inch thick, in an electric oven maintained at 124° C. where it is heated for twenty minutes. It is not necessary to open the oven until oxidation is completed if the flasks are thoroughly shaken before being put in.

On removal from the oven, further oxidation and destruction of reagent are prevented by adding 50 to 75 c.c. of ice cold distilled water, the flasks stoppered and set aside until ready to titrate. They are titrated with 0.1 N sodium thiosulphate after adding 10 c.c. of 10 per cent potassium iodide (freshly prepared), using a few drops of 1 per cent starch emulsion as an indicator.

If all reagents are pure and glassware clean, a blank need not be introduced until the stage where the silver chromate reagent and potassium dichromate are added. Here a blank glass stoppered Erlenmeyer flask is introduced and carried through with the sample. The titration of the sample subtracted from the titra-

tion of the blank gives the c.c. of 0.1 N thiosulphate, and by equivalent weights of 0.1 N potassium dichromate, due to the phospholipid present (usually 5 to 10 c.c. differences are obtained). The thiosulphate should be prepared some days or weeks prior to use and standardized at least every two or three weeks, especially if deteriorating rapidly. The titration difference, calculated in terms of 0.1 N potassium dichromate, is then divided by 3, then by the weight (in gm.) of the leucocytes extracted and multiplied by 100. The result indicates the number of milligrams of phospholipid present in 100 gm. of white blood cells.

The whole procedure may be readily completed in half a day and does not require the continuous attention of the analyst. There are several stages at which, if desired, the procedure may be interrupted for some time. These are (a) after alcohol ether is added to the leucocyte sand mixture, (b) when the extract is prepared and corked in a cleaned bottle, (c) at the stage of acetone magnesium chloride precipitation and (d) moist ether solution of the phospholipids, at both stages by tightly corking the tubes, (e) after the moist ether solution is in the oxidation flask and (f) after cooling the oxidation mixture with ice water and stoppering. At Stages a and b the procedure may be held for weeks or even months, at Stages c, d, and e overnight and at Stage f for not more than a few hours. The procedure may thus be conveniently fitted into a research or routine laboratory time table. When samples are allowed to collect at Stage b as many as 24 or 32 (multiples of 8 to fit the usual heavy duty centrifuge) may be conveniently analyzed in one day. It is not advisable to put more than 8 to 10 flasks in the oven at one time but the load of any oven may be found by trial. One blank will serve for each batch put into the oven or if heating is uniform, for all samples in which the same oxidizing reagents are used.

The above procedure has been repeatedly found by the author to give an experimental error of less than 10 per cent, the error in the phospholipid estimation itself being about 5 per cent (percentage standard deviation). For example, a sample of 400 c.c. of human blood was divided into 8 portions of 50 c.c. each. Each portion of blood was subjected to the above procedure from beginning to end. The resulting values for phospholipid in the white blood cells were 1,080, 1,210, 1,230, 1,260, 1,300, 1,330, 1,340, and 1,460 mg. per cent, respectively, giving a mean of 1,276 and a standard deviation of 115 which is 9.1 per cent of the mean. Clinically, variations from one patient to another and from time to time in the same patient are usually much greater than this and up to several hundred per cent where physiologic or pathologic changes have taken place. The author considers a variation over 15 per cent in the same patient as suggestive and significant but very seldom is it necessary to consider experimental error at all as the changes are usually quite beyond it.

Finally, for comparative purposes the phospholipid content of the white blood cells in normal individuals may be noted. Eight cases have been previously reported¹ as 457, 572, 643, 672, 747, 990, 1,164, and 1,168 mg. per cent, respectively. To these may be added the following determined at various times since: 505, 631, 747, 780, 820, 827, 868, 891, 905, 905, 920, 1,020, 1,040, and 1,290 mg. per cent, respectively. The mean of these 22 normal individuals is 844 mg. per cent with a standard deviation of 241 or 24 per cent of the mean. From these figures it may be calculated that statistically any value for phospho

the wheel exerts downward pressure on the door, and seals the crevices between the rim of the door and the gasket on the shelf of the facing. Likewise, in removing the door, a few right hand turns of the wheel release the arms from underneath the catches. The casting (*B*) can then be rotated until the arms are free of the catches and the door can be lifted off the opening.

A 1½ inch galvanized iron pipe (*P*) leads from the center of the convex end of the chamber to a Crowell rotary air pump, Type 4D. This pump is run by a 5 horse power electric motor, and has a capacity of 40.5 cubic feet of air per minute, which insures excellent ventilation for the occupants of the chamber. The pump and motor can be housed at some remote spot, wherever space is available. In our installation this machinery is housed in the basement of the building (see Fig. 4).

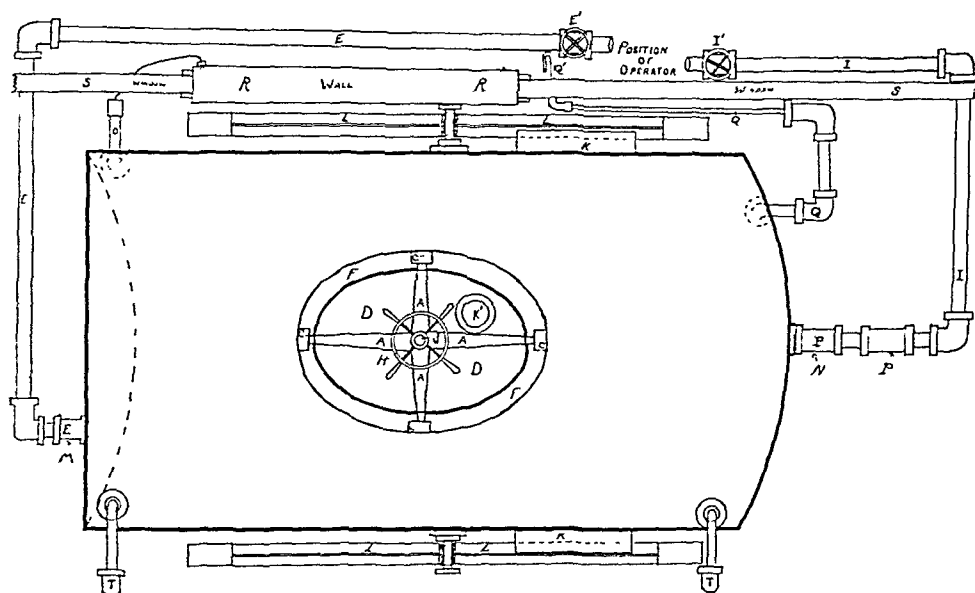


Fig. 1.—Top view of respiratory chamber.

Air is allowed to enter the chamber through a 1½ inch pipe (*E*), inserted in an upper lateral quadrant of the concave end. This position was chosen so that air might be drawn the entire length of the chamber, and further so that the occupants might assume a sitting posture without interfering with the entrance of air. This pipe is provided with an ordinary steam valve (*E'*), so that the amount of air entering the chamber is subject to easy control. There is a similar valve (*I'*), opening to the outside, in the pipe leading from the chamber to the pump. The purpose of this valve is to divert part of the suction produced by the pump, allowing the chamber to be decompressed slowly, so as to avoid any discomfort to the occupants.

The chamber is connected by means of a ¾ inch iron pipe (*Q*) and appropriate reducers, with a mercury manometer (*Q'*) attached to a meter stick, so that the total pressure within the chamber can easily be measured. As an added

vacuum it can be converted, by a few simple changes in the pump, into a pressure chamber. Such a pressure arrangement could be used either for experimental investigation or in therapeutics, as has been pointed out in the literature. Further, since it can be made gas tight, the chamber could be used for studying the effects of definite concentrations of various gases upon any animal organism. The value of the chamber is enhanced still further by the fact that, by a few simple additions, it would be possible to control, within narrow limits, the temperature within it.

It is quite possible that such a chamber, which can be subjected to rapid changes in pressure, would be of value in ear work; the opinion of an otologist would be of interest upon this point. The size of the apparatus, of course, would limit its use here.

The chief value of such a chamber lies, perhaps, in the fact that much research should be done upon the condition of anoxemia, owing to the popularity of airplane travel. The latter is becoming more and more important, and many individuals each year are subjected to a new physiologic environment. Commercial air transport companies find that it is best to fly at an altitude of 10,000 to 12,000 feet. A report of the Air Medical Service (1919) states that unacclimated persons are often sick at an altitude of 10,000 feet, and that many persons never become accustomed to an altitude of 14,000 feet. This would certainly indicate that much more work is needed upon the physiologic changes in anoxemia.

In summary: The principles of construction and operation of an apparatus by which human or large animal subjects can be subjected to reduced pressures are described. Some of the physiologic effects of lowered oxygen tension are mentioned, and other possible applications of such a chamber are pointed out.

REFERENCES

1. Kolls, A. C., and Loevenhart, A. S.: A Respiratory Chamber for Small Animals, *Am. J. Physiol.* 39: 67, 1915.
2. Van Liere, E. J., Crisler, G., and Robinson, D.: Effect of Anoxemia on the Emptying Time of the Stomach, *Arch. Int. Med.* 51, 796, 1933.
3. Crisler, G. R., Van Liere, E. J., and Wiles, I. A.: The Mechanism of the Delay in Gastric Emptying Time Caused by Anoxemia, *Am. J. Dig. Dis. & Nutr.* 2: 221, 1935.
4. Van Liere, E. J.: The Effect of Anoxemia on the Heart as Studied by X-ray, *Am. J. Physiol.* 82: 727, 1927.
5. Van Liere, E. J., and Crisler, G.: A Study of Vagospasm, *Am. J. Physiol.* 105: 469, 1933.
6. Van Liere, E. J., Crisler, G., and Hall, J. E.: The Effect of Digitalis on Acute Cardiac Dilatation Produced by Anoxemia, *J. Pharmacol. & Exper. Therap.* 52: 408, 1934.

A LABORATORY ELECTRIC FURNACE SUITABLE FOR MICRO KJELDAHL DIGESTIONS AND SIMILAR USES*

EDWARD B. SANIGAR, DR. NAT. SC., AND ALEXANDER J. ALLEN, PH. D.,
PHILADELPHIA, PA.

AFTER experiencing difficulties due to draughts and insulating deposits of soot when using gas heating for micro Kjeldahl digestions, attention was turned to electrical heating. The lack of suitable standard equipment made it necessary to design and construct a furnace especially for the purpose. A battery of four electric furnaces (enabling 24 digestions to be performed simultaneously) of the type described below was, therefore, devised. The furnaces are easy to assemble and dismantle (should the necessity of changing the heating coils ever arise), and have the heating coils entirely protected from draughts. They have proved to be very satisfactory, and embody a sufficient number of features of importance to suggest that a description of the assembly and its use will be of value to other workers.

Materials of Construction—

- $\frac{3}{4}$ in. asbestos board
- Sheet tin
- $\frac{3}{4}$ in. pine or deal board
- 4 way (off low medium high) reciprocating oven switch
- Nichrome wire, for heating units (Alloy V, 22 B & S, resistance 1 ohm/ft. Driver Harris Co.)
- $\frac{1}{2}$ in. asbestos tape
- 1 in. flat headed nails

Description—The sides, bottom and ends of the furnace are made of two thicknesses of the asbestos board, the inner pieces being cut smaller as required for proper fitting. This portion of the furnace is rigidly nailed together. The top of the furnace consists of one thickness of asbestos board, pierced by six 1-inch diameter holes (approx. $2\frac{1}{4}$ inches from center to center), and is kept in position by the sheet tin covering bent to cover the sides, bottom, and part of the top (see Fig. 1). The tin covering is kept in position by a tin binding strap ($1\frac{1}{2}$ inches in width) bent so that $\frac{3}{4}$ inch fits over the ends. A single nail, at the joining of the tin binding, is sufficient to keep the whole in position. The carrier for the heating units consists of a single thickness of asbestos board fitting loosely into the furnace, and which is nailed to transverse supports of asbestos board at each end and in two intermediate positions. If not adequately supported the carrier will sag, due to the heat, causing the coils to touch and short-circuit. The coils contain 20 feet of the nichrome wire which, before being stretched between the terminals at the ends of the furnace, was wound to a coil approximately $\frac{1}{2}$ inch in diameter and 4 inches in length. The terminals are ordinary $\frac{1}{8}$ inch diameter brass bolts and nuts.

*From the Cancer Research Laboratories, Graduate School of Medicine, University of Pennsylvania.

Received for publication September 26, 1935.

The assembled furnace is mounted on pieces of the asbestos board resting on $\frac{3}{4}$ inch thick wooden strips screwed to the baseboard, and is held firmly in place by two $\frac{1}{2}$ inch tin straps (screwed to the mounting) under which runs asbestos tape for heat insulation. A separate wiring diagram is not given since the actual connections will vary with the switch used. For high heat, the heating coils are connected in parallel; for medium, one coil alone is used, and for low, the two coils are connected in series. The two terminals at the end of the furnace not shown in the sketch are permanently connected by a short piece of $\frac{1}{2}$ inch brass strip, and one of these terminals is also connected to the black wire running between the two furnaces (see Fig. 1). By dividing the lead-in wires as indicated, one connection to the 110 volt a.c. supply serves two furnaces while still maintaining the independent operation of each. Ordinary, 18-gauge, twisted lamp cord is used for the wiring of the furnace.

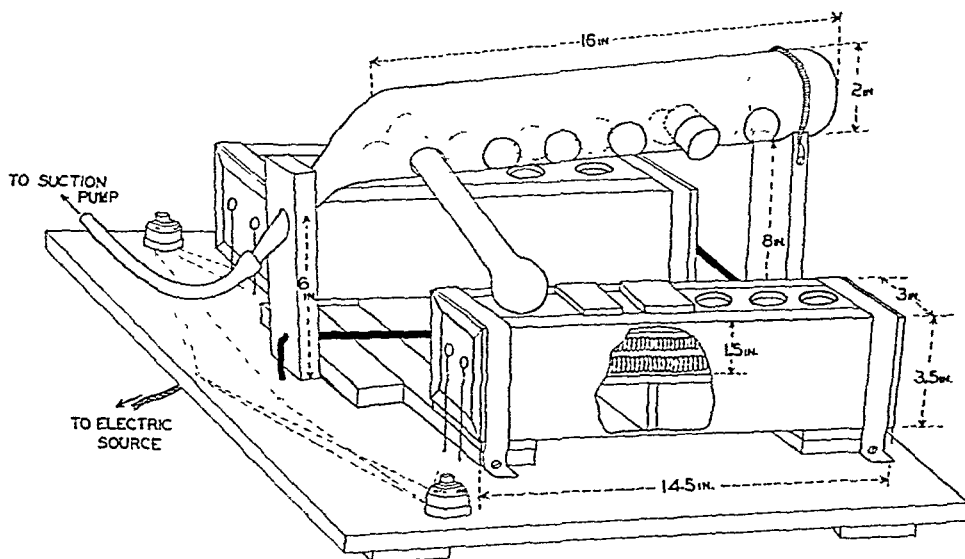


Fig. 1.

The exhaust system consists of a 2 inch diameter Pyrex glass tube closed at one end and drawn out at the other, and pierced with holes (of slightly greater diameter than the neck of the flasks used), at suitable positions relative to the holes in the furnace tops. The two furnaces emptying into the same exhaust are slightly staggered, as are the two sets of holes in the exhaust tube, so that the necks of the flasks being heated on the one furnace fall, in the exhaust tube, between those of the flasks on the companion furnace. The exhaust tube is mounted at an angle for drainage purposes, as shown, the closed end being secured by means of a spring passing around it, and is connected to a water suction pump. The heating apertures not in use are conveniently covered with small pieces of asbestos board, and the corresponding exhaust holes by means of corks.

One of us (E. B. S.) has used a battery of four such furnaces for Kjeldahl digestions almost daily for the past four months without any breakdown or accident, all four being frequently used simultaneously for seven or eight hours

continuously. They have proved to be very rapid, a total nitrogen microdigestion, using 0.1 cc of plasma, taking two to three hours on the average. No trouble has been experienced either with bumping or with the cracking of flasks due to condensate running down the outside of the digestion flasks.

The rubber tubings from the two exhaust tubes of the four furnaces in use at present are connected by a Y tube to a trap for the condensate, a wash bottle filled with concentrated sodium hydroxide solution (for further protection of the filter pump) and finally to a water suction pump. This has been found to remove satisfactorily all the fumes from the digestions, even though the apparatus is used on a bench in the open laboratory. The micro Kjeldahl flasks employed are very satisfactory, being made from 180 ± 0.5 mm outside diameter Pyrex tubing, 12 mm wall thickness, with a spherical bulb approximately 1.5 inches in diameter, the flasks having an overall length of about 7 inches.

A NEW METHOD AND A NEW PIPETTE FOR BLOOD SEDIMENTATION*

CLYDE BROOKS, M.D., NEW ORLEANS, LA

A NEW blood sedimentation method with a new pipette is described in this paper. It consists of using a straight glass tube, or pipette, of small uniform bore, by first filling it with an anticoagulant solution, and then blowing it out, leaving a thin film of the anticoagulant lining the inside or bore of the tube. Then the tube or pipette is filled by allowing the blood to flow into the pipette directly from the blood vessel of the patient, or else by sucking in the blood from a drop formed by pricking the finger, or any other suitable area of the body.

Then the pipette is set up in the vertical position, and the sedimentation of the red blood corpuscles is observed and recorded and plotted on a suitable chart.

The pipette consists essentially of a straight glass tube of about 1 to 1.5 mm bore. The pipette has a ground glass tip at one end, which fits into a hypodermic needle. At the other end of the glass pipette a short piece of rubber tubing is attached, which at the free end has a mouthpiece to be used for sucking fluid into, or blowing it out of the pipette. The pipette is divided into two unequal parts by a stopcock which cuts the lumen of the pipette precisely 100 mm from the extreme end of the ground glass tip, but cuts the lumen of the shorter limb about 30 mm from the end to which the rubber tube is to be attached. The longer limb of the pipette is graduated into millimeters, with zero at the extreme end of the ground glass tip,† and with the 100 mark exactly at the point where the lumen of the graduated limb is cut by the stopcock.

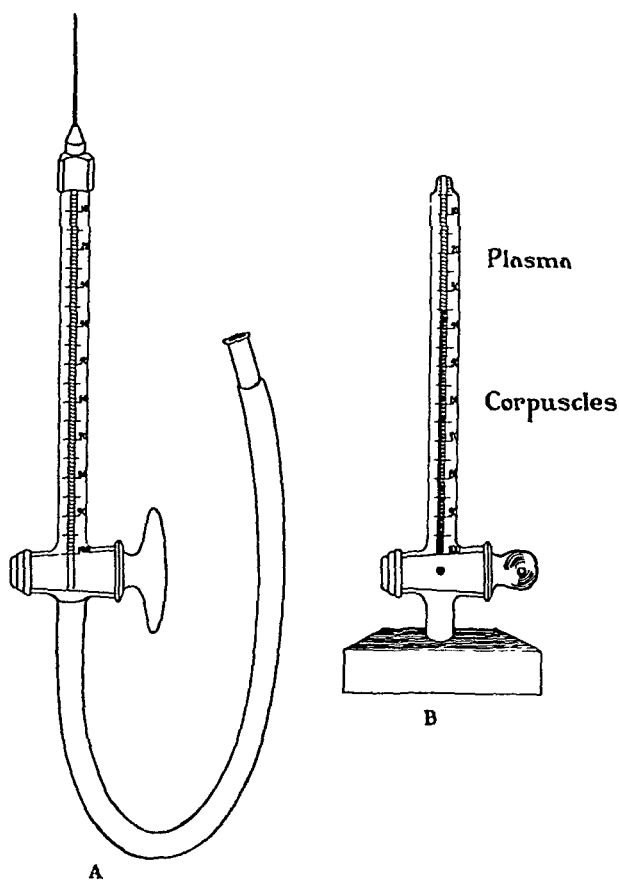
*From the Louisiana State University Medical Center.

Received for publication August 8, 1935.

†Though the zero point is at the extreme end of the tip there are no graduations marked on the tip because the engraving would weaken the tip too much.

In making the measurements of blood sedimentation rate, the following steps are performed as directed below:

1. First prepare a suitable anticoagulant solution, such as a sterile solution of 20 per cent potassium oxalate, put up in a sterile condition in a rubber capped 10 c.c. vial.



Blood sedimentation pipette

A - Ready to be filled

B - During sedimentation

Fig. 1.

2. Sterilize a blood sedimentation pipette, hypodermic needle which will fit the ground glass tip of the pipette, and also a short piece of rubber tubing with mouthpiece.

3. Adjust and fit the needle to the tip of the pipette, and attach the rubber tube with mouthpiece to the other end of the pipette.

4 Next sterilize the surface of the rubber cap of the vial of 20 per cent ovalate solution. Insert the needle which is attached to the tip of the pipette. Now, with the cock open, suck the ovalate solution through the needle and up into the bore of the pipette, filling it completely as far as, or a little beyond the 100 mark. Now turn the cock closing the lumen of the pipette, withdraw the needle from the vial of ovalate solution, and lay the filled pipette down on some sterile gauze.

5 Next prepare the patient's arm adjust a rubber band around the patient's arm rub some antiseptic over the skin covering the veins at the inner side of the bend of the elbow.

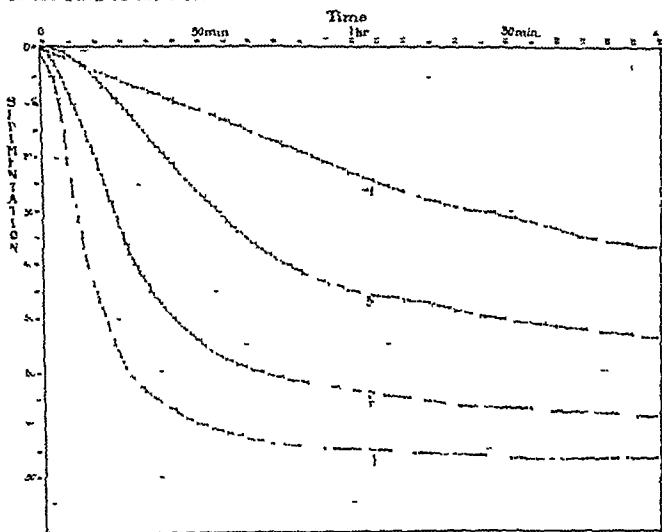


Fig. 2—Series of four sedimentation curves representing four different stages of the same case of severe lobar pneumonia of the left lung. 1, Extremely rapid sedimentation curve during the stage of complete consolidation. The patient is extremely ill. 2, Improved sedimentation curve (less rapid) during the stage of resolution. The patient is much improved. 3, Further improved sedimentation curve toward the end of resolution. The patient is about to leave the hospital although there is still an area of solid lung (unresolved) in the lower part of the left lung. 4, Normal sedimentation curve after the patient has completely recovered. Both lungs are clear.

6 Now open the cock of the pipette and blow out the ovalate solution, leaving a thin film covering the inside of the pipette. Then quickly insert the needle into the lumen of the vein and allow the blood to flow up through the needle into the lumen of the pipette, filling it completely as far as the 100 mark or a little beyond that point.

7 Next close the cock and withdraw the needle from the vein.

8 Turn the tip of the pipette upward in the vertical position, and, with a bit of gauze, remove the needle from the tip and wipe off the ground glass tip, leaving the upper meniscus of column of blood flush with the end of the tip.

This leaves the pipette completely and precisely filled from the very mouth of the tip (zero point) to the point where the stopcock cuts the lumen of the pipette (100 mark). Thus a column of fresh blood is cut off and held in the pipette, which column of blood is precisely 100 mm. in length. This measurement is automatically effected by closing the cock and removing the needle and wiping the tip.

9. Next immediately set the pipette up in a holder with the tip pointing vertically upward, and begin to observe the settling of red corpuscles, reading off the settling on the scale* and reading the time from a stop watch. Observe the settling, say every minute in rapidly settling cases, and every five minutes in slower ones. Keep up the observations for two hours. Record the results and plot the curves on coordinate paper.

In this method the blood is allowed to flow directly from the blood vessel of the patient into the pipette, where it is immediately set up and the observations of sedimentation at once begun. This means there is no dilution or mixing of the blood and no settling of the blood corpuscles before the actual observations have been started.

The stopcock automatically cuts off the column of blood precisely at the 100 mark and the removal of the hypodermic needle leaves the upper meniscus of the column exactly at the mouth of the tip of the pipette, which means the column of blood is exactly 100 mm. long. This measurement, being automatic, is always uniform and precise even in the hands of inexperienced laboratory workers. With this method the results of measurements or rate of blood sedimentation are so accurate that very slight alterations in blood sedimentation can be determined with certainty. By sterilizing the pipette and needle and rubber tube before filling the pipette, the observations can be continued for twenty-four hours or longer in order to determine the more complete sedimentation reading, without any decomposition occurring in the blood.

There is no possibility of leakage because the stopcock effectively closes the lower end of the graduated portion of the pipette. No air can get into the lumen of the pipette because the blood flows into the pipette under its own pressure. If the needle does not fit the ground glass tip quite nicely, there may be some blood leaking out; but no air will pass into the pipette and no air bubbles can get mixed with the blood.

Since the graduations on the barrel of the pipette are in millimeters, and since the graduated portion of the barrel is 100 mm. long, the graduations represent, not only millimeters, but they also represent percentage of settling of the red blood corpuscles. This is a convenience in making and calculating the readings and in plotting the curves showing the results.

The whole procedure is so simple and easy to perform and so precise in results, that it should be generally adopted and used by the medical profession.

*Since there are no graduations engraved on the ground glass tip, the measurements of settling in this part of the pipette must be read by either estimating the readings or by placing a millimeter scale beside the ground glass tip.

BOCK'S ERYTHROCYTOMETER AND TECHNIC FOR ITS USE*

E A SHARP, M D, AND E M SCHLEICHER, A B, DETROIT, MICH

BOCK¹ in 1933 described an inexpensive erythrocytometer employing the well known principle of halometry. This method for measuring blood cells is so well known through the work of Pippe, Eve, and others that comment is unnecessary.

Inasmuch as the Metz Echelon micrometer eyepiece (Leitz) was under study at the time Bock described his instrument, a comparative study of these two methods of erythrocytometry appeared attractive.

It will be seen by the diagram (Fig 1) that Bock's halometer is compact and simple in construction. It consists of three sections: the table (A) is superimposed on the outer supporting tube (B), within the main supporting tube is a movable, close fitting cylinder (C) controlled by a set screw (J) which can be released or secured during the mensural procedure, the third section serves as the supporting base (E) and lighting apparatus (F and G). The table has a central aperture (K) 1 cm. in diameter. The movable inner cylinder (C) is closed by a diaphragm at its lower end. Dual apertures (I) in the diaphragm, each 0.5 cm. in diameter, permit the two beams of light to pass through the object of study placed over the central aperture on the table. The light in the base is transmitted through a frosted glass plate (H).

The set screw, attached to the movable inner cylinder through a slot in the outer envelope, is used to control its vertical excursion within the outer envelope. On the opposite side from the set screw is another vertical slot in the outer envelope through which projects a pointer (D) attached to the inner sleeve. The pointer moves over a scale calibrated in 0.2 microns. The overall height of the apparatus is 38 cm.

Preparation of the Blood Film—In order to make erythrocytic observations uniform a standard blood smear is employed. The objective of the technique is to prepare the "margin free" smear of Schilling. The blood is taken from a finger tip, which is wiped off sharply with ether (not alcohol) to make it hyperemic. The skin is punctured about 5 mm. deep with a lancet (7 mm. long and 3 mm. in width, Eimer & Amend, New York). The first drop oozing from the puncture, or upon very slight pressure, is discarded with the aid of a clean gauze sponge. The second small drop is picked up with the edge of a No. 2 coverslip, 18 mm. size, the specimen edge of the coverslip being placed about one half inch from one end of the microslide at an angle of 45°. After allowing the blood to spread along the edge of the coverslip it is drawn toward the other end of the slide with

*From the Anemia Laboratory, Out Patient Department, Harper Hospital.
Received for publication August 12, 1935.

an even, firm and rapid movement, so that the blood follows without any crushing. A satisfactory preparation is made from a drop of blood that will make a smear extending one-third to one-half the length of the slide.

A "usable area" in the standard, unstained blood film is selected by microscopic examination. It is determined by the appearance of an area where the cell edges are touching or almost touching each other without any appreciable overlapping.

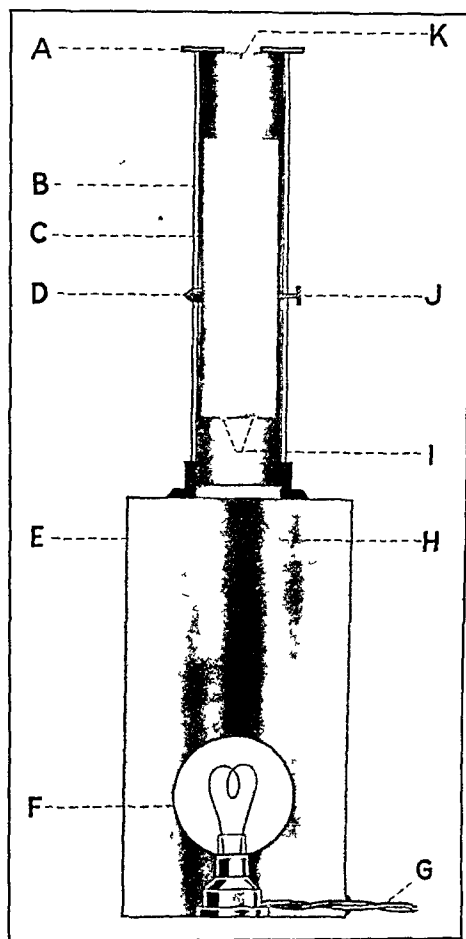


Fig 1.

The Determination of Mean Diameter of Erythrocytes by Bock's Halometer.—When a "usable area" has been found in the unstained blood preparation, the microslide is placed on the table of the erythrocytometer, film side up, so that the "usable area" completely covers the aperture. An unstained blood film on a high-grade glass slide shows two distinct, sharply defined, spectral halos within the movable cylinder. The outer red bands are used for measurement. A black line should not be present between the two red halos at the reading point, nor should there be any difference in the intensity of the colors of the contiguous red bands. By manipulating the set screw, lifting and depressing the

inner sleeve, two well defined red halos are brought together without overlapping, the set screw is secured and the measurement is taken from the scale

To insure greater accuracy of reading, record the initial measurement loosen the set screw, depress the inner sleeve slowly until two yellow bands touch each other, then slowly elevate the sleeve until the peripheries of the red bands are again in apposition This procedure can be repeated by elevating the sleeve to "gray" and lowering to the "red" again

Two blood preparations are always used for halometry Four readings on each film are taken from their respective usable areas The grand total of the readings in microns divided by eight represents the mean diameter of approximately one million erythrocytes

Range of Mean Diameters in Anemia—The mean diameter, determined by Bock's erythrocytometer on 44 relapsed cases of pernicious anemia, has ranged from 7.9 to 9.0 microns Compared with mean diameters on the same cases determined on 200 red cells measured by the Lehelon ocular micrometer the difference between the two calculations has been negligible There is, however, a great difference in the time required for the two procedures The mean diameter can be determined with a Bock instrument in two to three minutes, while the measurement of 200 cells with the ocular micrometer is well known as a time consuming procedure

It is noteworthy that there was no instance when the halometric mean diameter was at variance with the determination made by the ocular micrometer in the course of studying several thousand blood films One exception may be when extreme degrees of hypochromemia were present Faint halos result from deficient erythrocytic pigment, hence, experience is required to insure accuracy in extreme hypochromasia

A comparative study of stained and unstained preparations discloses that there is a slight difference in mean diameters taken on the unstained and the stained blood film, seldom more than 0.5 microns has been detected The lower reading is found in the stained preparation

It is believed, therefore, that Bock's erythrocytometer is a satisfactory instrument for the rapid determination of the mean diameter of red blood cells Comprehensive data on comparative mean erythrocytic diameters determined by the halometer and ocular micrometer on various anemias are being organized for a later communication

REFERENCE

- 1 Bock, H. E. Über ein neues Linfisches Erythrocytenmassgerät für Praktisch, Klin. Zwecke (Erythrocytometer) 12 1141, 1933

A GENERAL PURPOSE POLYCHROME BLOOD STAIN*

WILLIAM A. GROAT, B.S., M.D., SYRACUSE, N. Y.

THERE have been many excellent blood stains published and some of them, such as Jenner's,¹ Wright's,² and Giemsa's,³ widely used in the recent decades. All of these and most of the other good ones are in reality modifications of Nocht's⁴ and Jenner's¹ stains or embody the fundamental principles thereof. Romanowsky⁵ in 1891 had published some studies of the malarial organisms in which he seems to have been the first to use a mixture of eosin and methylene blue. Nocht⁴ in 1898 mixed Unna's polychrome methylene blue with eosin to obtain a mixture which was the first truly polychrome stain, but he did not know he had made new chemical compounds and did not separate the eosinates from the mixture. Jenner¹ in 1899 was the first to filter off the precipitate obtained by mixing aqueous solutions of eosin and methylene blue and to dissolve it in methyl alcohol for use as a blood stain. The May-Grünwald⁶ stain of 1902 is to all intents and purposes the same as Jenner's.¹ The Wright stain which is so universally used, and Hasting stain, which is practically identical with it, and other variants, named and unnamed, are modifications of the Leishmann⁷ stain. These Leishmann variants differ only in the manner in which the methylene blue is heated with a dilute alkali and the kind of alkali and the amount of heat used to form oxidation products. Giemsa thought to obtain some of the fractions of the eosinated polychrome methylene blue mixtures and recombine them in fixed proportions. The stain is good so far as it goes but he did not realize how many fractions there were. He made clear the importance of a methylene azure, fixing in the literature the words "azure stain," "azure granules," "azure chromatin," etc.; but he did not know there were at least three methylene azures and probably several more similar compounds to which reference will be made later. Some of the now less utilized staining mixtures, like Pappenheim's universal, were Jenner's or May-Grünwald combined with some one of the several Giemsa formulas. Kehrman⁸ in 1906 was the first to throw some real light on the chemistry and the complexity of the blood stains, and MacNeal⁹ in 1922 in his tetrachrome stain did put some of these facts together into a stain formula. Conn¹⁰ in his discussion of this stain sequence will be found most illuminating and was the source of some of these facts. The only reason for reporting another staining mixture would be to shorten the manufacturing process, simplify the technic of staining, improve the results, or for adaptation to some special use. The stain which I now report does somewhat simplify manufacturing and staining technic. It gives uniformly good results and has done so in various hands over a period of some twenty years. Recently it has been put to particular use in the photomicrography of blood.

*From the Department of Clinical Pathology, College of Medicine, Syracuse University.
Received for publication, August 3, 1935.

It seems to be well adapted to both black and white and natural color processes. The technical procedure and particular application of the stain in photomicrography will be given separately.

This stain has become rather widely used locally and as its use has been spread by graduates of the College of Medicine, Syracuse University, and in letters, numerous requests for the formula have been received.

The so called polychrome feature of the present day stains is a complex thing by no means dependent upon any single feature. The basic commercial stains are but mixtures, particularly the blues and violets, and the variations of the eosins cover a considerable range. When one makes a so called eosinate of methylene blue, he makes more than one eosinate, he not only makes several eosinates of methylene blue as chemical entities, but also makes eosinates of other basic dyes present in small amounts in the original methylene blue. Other variations are dependent upon the eosin used. The same is true of eosinates of methylene azure, methyl blue, methyl violet, and thionin. I became impressed with this fact through working with the various Giemsa stains and modifications. A reasonably pure eosinate of methylene azure giving certain very definite and well known so called azure qualities in nuclei and chromatin will lack some of the other characteristics of a so called panoptic stain. It would seem therefore that various combinations of the reasonably pure eosinates of methylene azure with other eosinates would produce a good general purpose stain. In the very generally used Wright's stain and in Hastings' stain and others of the Leishmann type, solutions of methylene blue are heated with a weak alkali to produce uncertain amounts of methylene azure. In some manner the alkali is then washed out or neutralized and an eosinate made. I became convinced that the variability in different lots of stain was due in part to slight changes in pH and in the amount of electrolyte present due to alkalization and neutralization. The workings of all textile and paper makers' staining methods suggest the importance of the electrolytes and colloidal dispersion in staining qualities and penetration. Hayden's blood method using a buffered dilution solution is a valuable contribution based on these principles. The method I devised for making stain took into account that we were dealing with mixtures to start with and that the more the blues were mixed within reasonable limits, and the more carefully one avoided the use of alkali or other additions which might disturb the ultimate balance, the better and more uniform the results would be. The necessary amounts of the various secondary constituents are small (Wilson¹¹). To laboriously and expensively separate and "purify" them only to recombine them is a waste of time and money. They can be obtained in reasonable amounts by mixing various commercial types. What is more, additional minor but useful and beautiful characteristics are unexpectedly obtained. The stain to be described takes advantage of these facts. Its manufacture and the technique of its use are both simplified. An extensive experience with students and technicians has shown it to be a sound routine stain. It is nearer foolproof than the more complicated ones and rather uniformly good results may be obtained by the unskilled. When most carefully used I believe extremely good results may be expected.

Only minor changes in the formula for this stain have been made over a fifteen year period of use. The present formula is a revision to meet the new

and more definite standards of the Committee on Standardization of Biological Stains. It has been thoroughly tested in its present form and used routinely for over two years, and we are convinced our manufacturing results are more uniform and the staining results have improved since using this stain.

GROAT'S MODIFICATION OF JENNER'S STAIN

No. 1

Eosin yellowish
Water and alcohol sol.
C. I. No. 768*
Total dye content 94 per cent 6.0 gm.
Distilled water 500 c.c.

No. 2

Methylene Blue U.S.P. medicinal
C. I. No. 922*
Total dye content 88 per cent 5.0 gm.
Methylviolet 2B
C. I. No. 680*
Total dye content 81 per cent 1.0 gm.
Thionin (Erlieh-Hoyer)
C. I. No. 920* 0.2 gm.
Distilled water 500 c.c.

*Certified by Commission on Standardization of Biological Stains, H. J. Conn, Chairman. National Aniline & Chemical Corporation certified stains were used by us.

Mix Solutions 1 and 2 and warm gently (use asbestos) to 50° C. and let stand twenty-four hours in incubator. Filter using hard filter paper, wash residue three times with cold distilled water. The last water should be pale blue. Dry thoroughly. Pulverize off paper.

Stain.—

0.5 gm. powder
100 c.c. Absolute Methyl Alcohol (chemically pure, free from acetone)
Stand two weeks, shake frequently.

The staining period is best determined by staining a normal smear. Improves with age if shaken occasionally. However, use only the clear supernatant fluid or filtrate.

METHOD OF USE

For a Single Slide.—Cover slide as fully as possible with undiluted stain and stain for an average of five minutes. Then plunge slide and stain thereon into 50 c.c. of neutral distilled water, mix stain and water by agitating the slide and then allow slide to rest vertically in the mixture of stain and water until it becomes rose pink. For coverslip spreads use proportionate amounts.

For Routine Laboratory Use.—Coplin jars may be used with pure stain in the first, diluted 1 to 10 stain in second, and third jar of clear, or nearly clear distilled water for final "pinking" of slightly overstained slides. Average time five minutes in first, five minutes in second, and rinse in third jar.

For Photography.—Purplish tints are required. Use fresh and filtered stains and dilutions, six minutes in first jar, fifteen minutes or more in the second jar (1-10 dilution) and quick rinsing in third jar (neutral distilled water) or until pink.

Stains vary in time required but wide latitude in routine work gives good results. For best effects slightly overstaining in the first jar with considerable prolongation of time in the second and possibly the third jars is recommended. Water freshly distilled once from permittit softened supply is recommended.

For Marrow.—Direct thin spreads of marrow from trephine biopsies or fresh autopsy material are made on slides. These are covered with pure dry ether for three minutes, again with fresh ether for three minutes and then with 95 per cent ethyl alcohol for three minutes and dried in air. They may then be stained as for blood for examination and for photography. Increase staining period 25 per cent or more if spreads are thick.

We hold with those who believe that marrow aspirations are more or less mixtures of circulating blood and marrow. However, smears made from such material may be stained like blood or marrow depending upon the character of the mixture.

When a marrow method involving emulsification of a bit of marrow in clear human serum is used, the spreads may be stained as blood, increasing the staining time unless very thin.

When this stain is used routinely the nuclei of the polymorphonuclear leucocytes are quite densely and clearly blue with fine detail. The nuclei of the large lymphocytes are pale purplish blue and of the smaller lymphocytes deep purplish blue. The nuclei of the monocytes are grayish blue with a distinct fine reticulum as contrasted with the coarsely lobulated and wavy chromatin pattern of the lymphocytes. Nuclei of normoblasts are bluish black, and of the younger erythroblasts variable paler shades of blue with good detail of the chromatin pattern depending upon maturity as in other stains. The neutrophile granules are a bright rose pink and stand out prominently. A rather remarkable variation in granule size and brilliancy brings out the various stages of maturity, the younger ones being more distinctly defined, varying from purplish to rose. The so called toxic reaction is well defined with blue granules very clearly shown. The neutrophile granules stand out more sharply than they do with an average Leishmann type stain. Eosinophile granules are slightly but definitely brighter and may be distinguished by their larger size. Basophile granules are purple. Fine dustlike, blue gray granules appear in mature monocytes. The so called azure granules in the various mononucleated cells are well shown. Cytoplasm of leucocytes varies in practically the same manner as in Wright's and other similar stains. Malarial parasites are shown quite definitely greenish blue with sharply defined chromatin of a rich pink to magenta. Auer bodies, Schuffner's dots and Cabot's rings are shown as in other panoptic stains and blood platelets are lavender with magenta chromatin. Erythrocytes are a bright rose pink. Polychromatophils are very well shown, both the diffuse and the punctate. Basophils of lead poisoning is clearly shown, the granules being a bright blue.

With more careful manipulation as described for photography, the fine staining of the granules can be maintained and at the same time various shades of purple may be emphasized in the lymphocytes and made to appear in the polymorphonuclear nuclei to the extent desirable for photography, while the nuclei of the monocytes remain more definitely bluish gray and erythroblast nuclei bluish black. All the good staining features of the red cells are maintained. Bluish, greenish, or washed out erythrocyte effects are avoidable in staining for parasites and platelets.

The comparative ease with which the stain may be made and used is indicated by the fact that at first we made the stain at a central laboratory and distributed it, but for some ten years now at the Medical College and at the Syracuse hospitals where this stain is used as a routine, the various technicians are making equally good batches of stain and have only minor difficulties in handling it. Internes take to it readily and continue to use it after they leave us. As already said, the good results obtained with it in photomicrography and the technical reasons for them will be given in a separate paper embodying also some of the technical features of blood photomicrography.

COMMENTS

Present knowledge of the chemistry of blood stains leaves much to be explained. There are three eosinates of methylene blue, at least three thionin azures, one or two methylviolets and undoubtedly numerous other eosinates in the mixtures commonly used. "Polychrome," rather than "trichrome" or "tetrachrome," is more descriptive for present-day blood stains.

The physical state, colloidal balance, and hydrogen ion concentration are potent factors in all staining and dyeing processes. With the best chemical and technical control, actual results cannot be wholly predicted. The textile does not match; the paper is mottled instead of uniform tint; the blood cells are "off color"; and often none can tell why.

The manufacturing method herewith described corrals as many useful ingredients as possible without undue expense, the product seems to have a sufficiency of them, and we avoid the known pitfalls of application and consolidate much of the valued experience of others into simple procedures.

REFERENCES

1. Jenner, L.: A New Preparation for Rapidly Fixing and Staining Blood, *Lancet* Pt. 1, 370, 1899.
2. Wright, J. H.: A Rapid Method for the Differential Staining of Blood Films and Malarial Parasites, *J. Med. Res.* 7: 138, 1902.
3. Giemsa, G.: Färbemethoden für Malariaparasiten, *Zentralbl. f. Bakt.* 31: 420; 32: 307, 1902.
4. Nocht: Zur Färbung der Malariaparasiten, *Zentralbl. f. Bakt.* 24: 839, 1898.
5. Romanowsky, D.: Zur Frage der Parasitologie und Therapie der Malaria, *St. Petersburg. med. Wehnschr.* 16: 297 and 307, 1891.
6. May, R., and Grünwald, L.: Über Blutfärbungen, *Zentralbl. f. inn. Med.* 23: 265, 1902.
7. Leishmann, W. B.: A Simple and Rapid Method of Producing Romanowsky Staining in Malarial and Other Blood Films, *Brit. Med. J.* Pt. 2, 757, 1901.
8. Kehrman, F.: Über Methylen-azur, *Ber. d. deutsch. chem. Gesellsch.* 39: 1403, 1906.
9. MacNeal, W. J.: Tetrachrome Blood Stain; an Economical and Satisfactory Imitation of Leishmann's Stain, *J. A. M. A.* 78: 1122, 1922.
10. Conn, H. J.: History of Staining. The Staining of Blood and Parasitic Protozoa. *Stain Technology* 5: 127, 1930.
11. Wilson: *J. Exper. Med.* 9: 645, 1907.

DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILBUFFE, M D, ABSTRACT EDITOR

ANEMIA of Premature Infants, Abt, A F. Am J Dis Child 49. 1204, 1935

In the series of premature infants here reported on, the values for whole blood iron closely approximated the values for hemoglobin

On comparing the curves for the whole blood iron and the hemoglobin values in the series of premature infants with similar curves for full term infants within the first eight months of life, it was found that the values are equally high at birth and the rates of fall in the two groups are parallel, the lowest values are reached at about the twelfth week of life. The types of the curves are similar, the chief difference being that in premature infants the hemoglobin and the blood iron values reach lower levels at corresponding age periods.

The factor

$$\frac{\text{Hemoglobin in percentage}}{\text{Whole blood iron in mg per hundred cc}}$$

determined by Kotikoff for full term infants is practically the same as that here reported for premature infants

It has been noted that certain common factors produce the anemia of the full term as well as that of the premature infant during the early months of life. These are the change from a low intrauterine oxygen tension to the higher postnatal tension of atmospheric air, the increased hemolysis of erythrocytes after birth, the fragility of the immature red blood cells of the newborn, and the embryonal type of hemoglobin, different chemically and spectroscopically from the hemoglobin of older infants

The premature infant differs, in part at least, from the full term infant because he has a relatively more rapid growth, and he should therefore have a more rapidly increasing blood volume with a greater demand for formation of blood, a more immature and probably less efficient hematopoietic system (functional incapacity), and a smaller supply of blood to begin with, which might be expected to yield a more limited amount of blood building materials. The greater decline in the values for hemoglobin and for whole blood iron in the premature infant as compared with those in the full term infant may be explained by these physiologic factors which are peculiar to prematurity

SYPHILIS, and Pregnancy, a Clinical Study of 2,150 Cases, Mc Cord, J. R. J A M A 105: 89, 1935

Pregnancy does not affect the reliability of the Wassermann reaction.

This test should be a routine part of antepartum care

Regardless of the activity of the disease, sufficient antepartum antisyphilitic treatment assures the woman a syphilis free baby in 95 per cent of the cases

The best results will be obtained with ten or more treatments

Treatment should be mild but continuous and should not be controlled by the Wassermann reaction

The concurrent use of arsenic and a heavy metal has worked well

Such therapy seems to be safe for the mother

In the vast majority of cases, a strongly positive cord Wassermann test, properly done, means that the baby has congenital syphilis. A negative cord Wassermann reaction is of little value in the diagnosis of congenital syphilis

The characteristic picture of osteochondritis of the long bones is pathognomonic of congenital syphilis

There seems to be no condition in medicine that returns such huge dividends in life and health with such a small output of energy and money as that seen in the prevention of congenital syphilis.

LEUKEMIA: Its Diagnosis and Treatment, Rosenthal, N. J. A. M. A. 104: 702, 1935.

The relative occurrence of the three principal groups of leucemia apparently corresponds with the relative percentage of the various types of leucocytes in the circulating blood: namely, granulocytes, lymphocytes, and monocytes.

The underlying systemic disorders present in all cases is essentially the same. An arbitrary division may be made according to the duration of the disease, acute or chronic, and also according to the number of white blood cells, into leucopenic and leucocythemic forms.

Symptomatology, although of great value in differentiating the disease, is unreliable for purposes of diagnosis. This should be based on the characteristic blood changes, which do not depend so much on the number of white blood cells as on the presence and persistence of specific types of cells, such as myelocytes, myeloblasts and relative and absolute lymphocytosis.

Confirmatory diagnosis of the more obscure varieties may be made by biopsy on the sternal bone marrow or on a lymph node.

The treatment of leucemia is largely symptomatic. Arsenic, transfusions and particularly roentgen irradiations are the chief means of inducing symptomatic improvement, remission, or possibly prolongation of life.

LEUKEMIA: Differential Diagnosis of the Leukemic States, Kracke, R. R., and Garver, H. J. A. M. A. 104: 697, 1935.

There still remains no unanimity of opinion relative to the origin of white blood cells, but it seems probable that under normal conditions myeloid cells arise mainly from the bone marrow and lymphoid cells from the lymphoid glandular system, including the spleen.

It is virtually impossible to distinguish between the various types of leucoblasts with ordinary staining methods, or even with vital staining.

Cases of chronic leucemias can be diagnosed easily as well as acute forms, provided the total white cell count is definitely increased with a preponderance of blast cells.

The leucemic states in which the total number of white cells is normal, or below normal, offer considerable difficulty in diagnosis both clinically and hematologically and are likely to be confused with various leucopenic diseases.

The most reliable criterion for the diagnosis of any leucemia is a preponderance of immature cells regardless of the total number. Studies on a large number of cases of monocytic leucemia that are filed in the Hematological Registry indicate that the chief cell type has its origin in the bone marrow and adds further evidence that monocytic leucemia is but an atypical phase of myelogenous leucemia.

MYELOMA, Bence-Jones Proteinemia in Multiple, Cantarow, A. Am. J. M. Sc. 189: 425, 1935.

A case is reported of multiple myeloma (granulocytic type) with severe anemia and renal failure. Bence-Jones protein was present in the urine and was also demonstrated in the blood serum.

The total serum protein concentration varied between 6.84 and 11.3 gm. per 100 c.c., the Bence-Jones protein being approximately 3 gm. when the total protein was 8.7 gm. and 5.2 gm. when the latter was 11.3 gm. per 100 c.c.

Hyperealcemia (12.9 mg. per cent) was present at the time of admission, the serum calcium concentration falling to 9.2 mg. per cent while the serum inorganic phosphorus concentration increased from 5.8 mg. to 12.5 mg. and the blood creatinine from 6.1 mg. to 13.6 mg. per 100 c.c.

The relatively rapid and marked fluctuations in serum protein and serum calcium concentrations in patients with multiple myeloma and the variability of the several factors which determine the characteristic precipitation reactions of Bence-Jones protein emphasize the necessity for repeated studies in such cases.

TUMORS, Malignant Epithelial, of the Neck, Oliver, R L Am J Cancer 23 16, 1935

A study of 80 cases of carcinoma arising in the deep tissues of the neck without relation to the epidermis or glandular organs is presented. The relationship of these tumors to the development of the branchial clefts is discussed, and the embryology of these structures, which form the basis for the origin of the tumors, is outlined.

Among the 80 cases, males predominated in the ratio of nine to one, and the peak of age incidence was in the sixth decade. The average duration of symptoms was between six and seven months. The usual symptom of onset was the appearance of a tumor in the neck without relationship to clinical findings in the throat or in other organs in the cervical region. Stiffness of the neck, headache, hoarseness, pain, and general cervical node enlargement were among the other clinical findings. In about 10 per cent of the cases trauma marked the onset of the clinical history. The position of the tumor was usually in the upper anterior cervical triangle, behind and below the angle of the jaw.

Only radical surgery sufficed as an attack upon these rapidly growing and infiltrating tumors. The usual operation advocated involves the block dissection of the glands of the neck with resection of the larger vessels of the neck, including in some instances the internal and common carotid artery.

Pathologically the tumors were grossly either solid or cystic, under the microscope they could be divided into squamous, cuboidal, and basal cell forms. The cuboidal and basal cell forms were subdivided into two groups: diffuse and alveolar.

In the differential diagnosis it was found necessary to rule out cervical lymphadenitis, benign branchial cleft cysts, malignant lymphomas, and metastatic carcinoma from the nasal sinuses or structures of the throat.

A consideration of the ultimate results in relation to the modes of treatment and the types of cellular pathology justifies the conclusion that these tumors, which are usually fatal (77 out of 80 cases), are best treated by radical surgery if seen in the earlier stages. They comprise a single pathologic entity grading from the less differentiated basal cell forms through the cuboidal cell type to the most highly differentiated squamous cell form. Surgery is slightly more favorable in these last two forms than in the first. Irradiation is probably advisable in conjunction with surgery in all forms, but particularly in the basal cell type. In advanced and inoperable cases palliative irradiation may be helpful.

TISSUE Preservation and Microscopic Examination of Nodules in Gout, DeGalantha, E Am J Clin Path 5 159, 1935

1 Fix fresh material immediately in absolute alcohol. Two changes of four hours each for thin specimens, longer (to twenty four hours) for thicker specimens.

2 Xylol paraffin (equal parts xylol and paraffin kept at 57°) for two hours.

3 Hard paraffin (melting point 56°) for one hour and embed.

4 Cut sections about 8 microns thick, fix to slide and dry in oven at 37° C for two hours.

5 Remove paraffin with xylol and then wash in absolute alcohol.

6 Stain three minutes in Harris hematoxylin.

7 Wash in absolute alcohol and counterstain with 2 per cent alcoholic eosin one minute.

8 Clear in several changes absolute alcohol, carbol xylol and xylol.

9 Mount in xylol Canada balsam.

For specific demonstration of urate crystals, use above technique through stage 5, then:

6A Place slides in 20 per cent silver nitrate solution and expose to strong sunlight until the urates are a bright rose color (one to four hours).

7A Prepare solution of (a) 10 c.c. 3 per cent gelatin in hot water, (b) 3 c.c. 20 per cent silver nitrate and (c) 2 c.c. 2 per cent hydroquinone, and pour this over slides until the urates turn black and the connective tissues are yellow.

8A Wash quickly in hot water (57° C).

9A Clear in absolute alcohol and xylol as in stage 8 above.

10A Mount in xylol Canada balsam.

For decalcifying specimens containing bone: (1) Place thin specimens in absolute alcohol containing 2 per cent concentrated nitric acid. Change to fresh solution every twenty four hours, until the bone is softened. (2) Wash in several changes of absolute alcohol for twenty four hours. (3) Embed and handle as above.

BLOOD CALCIUM, Chemical Estimation and Significance of, Mc Lean, F. C., and Hastings, A. B. *Am J. M. Sc.* 189: 601, 1935

The total calcium of the serum or plasma is nearly all accounted for as calcium ions and calcium bound to protein. Of these two forms the ionized calcium is of primary physiologic and clinical importance.

The calcium ion concentration of the plasma is normally maintained within a relatively narrow range by a process of physiologic regulation in which the parathyroid glands play a prominent part. This range has been found, as a rule, to be from 4.25 to 5.25 mg. per 100 cc.

The calcium ion concentration of the plasma, at any one time, is the resultant of an equilibrium between the total calcium and total protein present in the plasma.

A method for clinical estimation of calcium ion concentrations, by calculation from total protein and total calcium concentrations, is presented. Results obtained by this method have been found to be in substantial agreement with the results obtained by direct observation.

Fluctuations in the concentration of total calcium, occurring in response to fluctuations in the concentration of total protein in the plasma, are necessary for the maintenance of normal calcium ion concentrations, and consequently are not in themselves of clinical importance.

An increase in the calcium ion concentration in the plasma is presumptive evidence of hyperfunction of the parathyroid glands.

A decrease in the calcium ion concentration in the plasma may occur as the result of hypofunction of the parathyroid glands, or may be brought about by the hyperphosphatemia of uremia.

In all other conditions investigated, including various disorders of calcification, the calcium ion concentration of the serum or plasma has been found to be within normal limits.

BLOOD GLUCOSE CLEARANCE, Determination by a Microinterval Method. I. Studies in Normal and Diabetic Persons, Mc Kean, R. M., Myers, G. B., and Von Der Heide, E. C. *Am J. M. Sc.* 189: 702, 1935

The authors feel that a "normal" microinterval curve (peak of 175 mg. per cent or below and fifteen minute level of 125 mg. per cent or below) is strong presumptive evidence against the presence of diabetes mellitus. On the other hand, an abnormal curve is not of equal diagnostic significance. Curves similar to those obtained in diabetes have been found in several other conditions, such as cardiac decompensation, hypertension, cholecystitis, peptic ulcer, carcinoma and chronic encephalitis.

The fate of the injected glucose has been conjectured and an attempt has been made to explain the early appearance of a distinctive response in normals and diabetics.

PREGNANCY, Chemical Test for, Mencken, J. G. *Deutsche med. Wchnschr.* Leipzig 60. 1837, 1934.

Mencken found the test devised by Vischer and Bowman of value. The method follows:

Reagents—(1) 0.5 per cent solution of hydrogen peroxide. (2) 1 per cent aqueous solution of phenylhydrazine hydrochloride. (3) 5 per cent aqueous solution of methyl cyanide. (4) Concentrated hydrochloric acid. The reagents should be freshly prepared each week.

Method—(1) To 1 cc. of urine add 1 drop of peroxide solution, 5 drops of phenylhydrazine solution, 5 drops of methyl cyanide solution, and 5 drops of HCl. (2) Place the tube in a boiling water bath for twenty five minutes.

Reading—Positive russet color and flocculent precipitate Mencken usually obtained a dustlike and occasionally a somewhat more dispersed precipitate

Negative straw color and no or only a powdery precipitate Mencken found negative reactions light and positive reactions dark in color

PEPTIC ULCER Gastric Mucin Treatment of, Fogelson S J Arch Int Med 58 7, 1935

A study based on questionnaires concerning 494 patients with peptic ulcer treated by clinicians throughout the United States has demonstrated the ability of gastric mucin to control all the symptoms in 70.5 per cent and to afford a partial relief of symptoms in 23 per cent, while failure to afford any relief occurred in 6.5 per cent

In 217 patients with intractable ulcers who could not be relieved of symptoms by medical management, of whom 19 had been submitted to previous surgical procedures, gastric mucin afforded complete relief in 63.1 per cent, partial relief in 29.4 per cent, and no relief in 7.5 per cent

The results obtained in this group of patients with intractable ulcers suggest the possibility of obtaining symptomatic relief with gastric mucin in a relatively high percentage of patients in whom accepted orthodox measures, including operation, have failed

The permanence of the results is not considered, owing to the limited periods of observation

TUBERCULOSIS Renal, Spontaneous Healing of, Keyes, E L J A M A 104 16 1380

Medlar has shown that renal tuberculosis begins as a nonsurgical lesion that frequently heals

This lesion may be identified clinically as a tuberculous bacilluria (as defined in the text)

Surgical renal tuberculosis, characterized by gross changes shown by pyelography, is clinically a progressive disease with a fatal termination unless interrupted by nephrectomy

The pyelogram discloses surgical tuberculosis The earlier the nephrectomy, the greater the probability of cure

The healing of surgical renal tuberculosis by pathologic nephrectomy is extremely rare and, even with the kidney function gone, active tuberculosis may persist

Though surgical renal tuberculosis may remain latent for long periods of time latency is extremely rare and even in its most complete form, i.e., latency due to complete physiologic destruction of the kidney, the tuberculosis may still actually be active

The curative treatment of renal tuberculosis, old and new, is nephrectomy

WEIL'S DISEASE Serological Diagnosis of, Brown H C Brit M J 1 411, 1935

The procedure following, the "adhesion test," has been found as reliable as the agglutination test in the diagnosis of infection with *L. icterohemorrhagiae* It is also more rapidly performed and easier to read

The agglutination test (Schuffner) is performed by allowing various concentrations of serum to interact with an equal volume of a young culture for two hours at 32° C After incubation small drops from the various dilutions are placed on slides and examined, without a cover glass, by dark field illumination

The technique of the adhesion test follows

The following reagents are required (1) The patient's serum, (2) a young broth culture of *L. icterohemorrhagiae*, (3) a saline suspension of a young culture of *B. coli* or other similar organism (4) a fivefold dilution in saline of fresh guinea pig serum (this is not required if the patient's serum is tested on the same day the sample is taken)

One volume (about 20 cmm) of each of the above reagents is placed in a small agglutination tube and the contents mixed A control tube contains known normal serum in place of the patient's serum The tubes are incubated at 37° C for thirty minutes, and then a small drop is placed on a microscopic slide, covered with a coverslip, and examined by dark field illumination In the event of a negative reaction the leptospira will be seen swim

ming freely and totally unimpeded by the presence of bacteria. In a positive reaction the bacteria will be seen to be firmly adherent to the leptospira. Not all the leptospira will be affected in this way, but only a certain proportion, unless the serum is of a very high titer.

At least twenty leptospira should be observed before a negative result is recorded. It may be that one or more bacteria, in a positive reaction, will adhere to the end of the organism, and no amount of movement on the part of the leptospira will dislodge them. Other leptospira will be seen almost completely covered with bacteria, with only the terminal portions still in sight. The reaction is very dramatic, and no difficulty should be experienced in reading the results.

What occasionally happens with a negative serum is that a leptospira may be seen more or less in the center of a small clump of bacteria. In this case it will be noticed that it is not adherent to the bacteria, but slides in and out of the bacterial clump, a totally different state of affairs from that in which there is true adhesion. When there is a positive reaction with the patient's serum a second test can be made, using dilutions of serum which will give total concentrations up to 1 in 30,000.

The reaction is marked by the end of the second week and may be expected to appear approximately as soon as the agglutination test (sixth day).

The culture medium used in the adhesion test is a modification of that recommended by Fletcher (1927), and is as follows: distilled water, 3 c.c.; lemco broth, 0.5 c.c.; inactivated rabbit's serum, 0.25 c.c. The culture is incubated at 32° C. for six days. It is preferable to use a strain which has lost its pathogenicity.

PNEUMONIA, Streptococcus, A Note on Parenteral Liver Therapy in, Wilson, J. A. Am. J. M. Sc. 189: 374, 1935.

The injection of liver extract was tried in the first case of pneumonia because of the drop in leucocyte count from 16,000 to 8,200 in three days. When liver extract was started, this patient was seriously ill, in a light stupor, high fever, able to take very little nourishment, and voided 500 c.c. of urine in twenty-four hours. The prognosis seemed poor. Sixteen cubic centimeters of liver extract (equivalent of 800 gm. of fresh liver) were given intramuscularly between 4 P.M. of the fifth day and 4 P.M. of the sixth. The leucocyte count rose from 8,200 to 27,800. The patient improved. He voided 3,400 c.c. of urine in twenty-four hours, and although still confused mentally the stupor was less and the fever lower. The leucocytes remained about 20,000 until the tenth day of the illness. The fever came down by lysis and there were no complications. He was discharged from the hospital on the fifteenth day.

The leucocyte count in the second case, on the third day of illness was 10,400 and had dropped to 5,200 on the fifth day. Daily liver extract injections were started on the fifth day and given for 5 days. The general condition of the patient was greatly improved and the average leucocyte count was 7,000. No injections were given on the tenth, eleventh, and twelfth days of the disease. The leucocyte count on the twelfth day was 4,600. Four cubic centimeters of liver extract were given on the thirteenth day, and on the fourteenth day the count rose to 12,200. In three more days the count dropped to 7,000. The temperature was normal by this time, the patient improved rapidly and no complications occurred.

Differential counts coincided with the number of leucocytes. An increased leucocyte count was accompanied by a relative increase in the neutrophils. The number of white blood cells seemed to be a sufficient index of the patient's condition.

In studying the hospital chart, it was interesting to note that the output of urine was below normal just before the liver therapy was instituted, and greatly increased just after it was given in each case. The first patient averaged an intake of 3,000 c.c. of fluids a day and the second 2,500 c.c. daily.

The Journal of Laboratory and Clinical Medicine

VOL 21

JUNE, 1936

No 9

Editor WARREN T VAUGHAN, M D

Richmond, Va

ASSOCIATE EDITORS

DENNIS E JACKSON, M D	- - - - -	CINCINNATI
W C MACCARTY, M D	- - - - -	ROCHESTER, MINN
GERALD B WEBB, M D	- - - - -	COLORADO SPRINGS
VICTOR C MYERS, Ph D	- - - - -	CLEVELAND
RUSSELL L HADEN, M D	- - - - -	CLEVELAND
JOHN A KOLMER, M D	- - - - -	PHILADELPHIA
ROBERT A KILDUFFE, M D	- - - - -	ATLANTIC CITY, N J
GEORGE HERRMANN, M D	- - - - -	GALVESTON
T B MAGATH, M D	- - - - -	ROCHESTER, MINN
DEAN LEWIS, M D	- - - - -	BALTIMORE
M H SOULE, Sc D	- - - - -	ANN ARBOR, MICH
CLIFFORD J BARBORKA, M D	- - - - -	CHICAGO

Contents of this Journal Copyright, 1936, by The C V Mosby Company—All Rights Reserved
Entered at the Post Office at St Louis, Mo., as Second Class Matter

EDITORIAL

RENAL FUNCTION TESTS

LABORATORY methods for the estimation of the type and amount of damage that has been done to diseased kidneys are being constantly improved. Classifications of nephritis have been simplified to conform with the data that can be clinically established. Clinical diagnosis can no longer be satisfied with mere qualitative estimation of the presence or absence of albumin and casts in the urine, along with blood pressure determinations or studies and clinical observations. As Addis and his assistants¹ have pointed out, the rate of elimination of protein or serum albumin in the urine as determined by quantitative estimations, along with the quantitative determination of the rate at which formed elements are passed in the urine, is necessary for satisfactory diagnosis and prognosis in renal disease.

Modern tests for the functional capacity of the kidneys will yield information of value not only in the diagnosis and prognosis but also information that is indispensable in the scientific treatment of patients with renal disorders. The

ability of the kidney to concentrate urine specimens, to excrete phenolsulphonephthalein and urea along with the maintenance of normal levels of nonprotein nitrogen waste in the blood plasma, are used in the establishment of renal function. Chapman and Halstead,² MacKay and Rytand,³ Alving and Van Slyke,⁴ Freyberg⁵ and Stieglitz⁶ have made interesting comparative studies of the value of various types of tests. The Van Slyke urea clearance test is generally accepted as the most accurate and most delicate method. It is a standard with which all other tests should be compared, but it requires facilities for microchemical blood and urinalysis.

Alving and Van Slyke⁴ have found that the *urinary specific gravity and volume output tests* compare favorably with the urea clearance, provided the tests are made under standard conditions. These simple tests, which have been variously named according to the authors of the test or a modification, as the Volhard, the Addis, the Hedinger and Schlayer, the Mosenthal, are more appropriately designated as the two-hourly renal, the urinary specific gravity, or the concentration and dilutions tests.

If albumin is found in any urine specimen, it should be quantitatively estimated by the Shevky and Stafford⁷ modification of the Esbach method, using Tsuchiya's reagent in graduated centrifuge tubes. If there is less than 1 gm. of albumin per liter the effect upon the specific gravity will be negligible. However, every 2.5 gm. of albumin add one point to the third decimal of the specific gravity. Precautions and care are necessary in the taking of the specific gravity.

The two hour and the quarter hour phenolsulphonephthalein excretion tests as introduced by Rowntree and Geraghty⁸ have been found by Christian⁹ and others to be of limited value, in that they fail to detect or show decreased excretion in the milder cases of nephritis with slight or moderate degrees of impairment. It is often normal after half the functional renal tissue has been destroyed. Furthermore, liver diseases were found to interfere by contributing unreliable, often high, excretion values. Chapman and Halstead² recently re-discovered the fact that the curve of elimination of phenolsulphonephthalein was slow to reach the maximum in nephritis and that a delay in the excretion is one of the earliest indications of impaired renal function. The normal 40 per cent was to be excreted within fifteen minutes after intravenous injection. In a large series of cases it was found that in the presence of nephritis there was invariably a decreased fifteen-minute output of the dyestuff and a marked abnormality in the curve of excretion, particularly a delayed peak in the elimination, even when the two-hour output was normal. The technic for performing the test is the same as the usual two-hour test, except that the dye in 1 c.c. amount, containing exactly 6 mg. measured in a tuberculin syringe, is introduced intravenously. The patient is asked to void immediately and drink 600 c.c. of water one-half hour before the injection. A specimen of urine must be obtained at a fifteen-minute interval even if catheterization must be resorted to. Then an hour specimen and again a second hour specimen as the usual test must be obtained. Concentration of the dyestuff in the fifteen-minute, one-hour, and two-hour specimens is determined in a colorimeter, using a standard solution of freshly prepared phenolsulphonephthalein.

Stieglitz and Knight¹ have recently offered as a substitute for the phenolsulphonephthalein test a *sodium ferrocyanide test*, in which the latter substance is introduced intravenously and the output determined quantitatively in the urine. The ferrocyanide is apparently filtered only through the glomeruli. The test, therefore, serves to detect the slightest of pathologic changes in the glomerular membrane. The test seems to have very definite advantages over the phenolsulphonephthalein excretion test, but further studies are necessary to establish the advantages and disadvantages.

Blood and Urine Chemical Tests of Kidney Function—Facilities for the prosecution of the methods of quantitative blood and urine chemistry are not always available for the general practitioner, but the rise and development of the clinical laboratory in most communities makes it possible for most physicians to avail themselves of these methods of precision. The total blood nonprotein nitrogen levels or those of any one of its constituents as urea, uric acid, creatinine, amino acid, or test nitrogen have long been considered indices of renal function. The percentages of the total nonprotein nitrogen that a constituent as urea makes up, or the ratio between the two, have been considered by German and French investigators to vary in the presence of renal impairment. Mosenthal and Bruger¹⁰ have recently brought forth substantiating evidence

The blood urea nitrogen—nonprotein nitrogen ratio, $\frac{\text{BUN.}}{\text{NPN}} \times 100$ normally remains approximately constant at about 44 or less according to the studies of Mosenthal and Bruger¹⁰. The ratio rises when renal impairment increases and reaches a level of 80 or more with a maximum impairment as a result of destruction of kidney tissue that has been accomplished. Improvement of the kidney function is evidenced by a drop in ratio, while fixation at a level above 50 and a rapidly rising ratio are to be considered as ill omens. The results of Mosenthal and Bruger¹⁰ compare favorably with the results of the urea clearance tests of Van Slyke. The advantages cited for this test over that of the urea clearance is that only one specimen of blood is necessary and the somewhat troublesome and sometimes difficult collection of urine specimens under basal conditions may be dispensed with.

A review of these modern methods of determining the functional state of the kidneys and the comparative studies and our own experiences leave us with the impression that the simple methods carefully carried out are well worth while. When possible, all of the methods outlined above should be applied and repeated at intervals where there is any question of impairment of the kidney function.

REFERENCES

1. Addis, T., and Sheikh, M. C. A Test of the Capacity of the Kidney to Produce a Urine of High Specific Gravity, *Arch. Int. Med.* 30: 559, 1922.
2. Gibbons, H. A Rapid Quantitative Method for Examining the Urine in Renal Disorders, *Arch. Int. Med.* 54: 758, 1934.
3. Chapman, E. M., and Hulstead, J. A. The Fractional Phenolsulphonephthalein Test of Bright's Disease, *Am. J. M. Sc.* 186: 223, 1933.
4. MacKay, E. M., and Rystrand, D. A. Significance of Phenolsulphonephthalein Test of Renal Function, *Arch. Int. Med.* 55: 131, 1935.
5. Alving, A. S., and Van Slyke, D. D. Significance of Concentration and Dilution Tests in Bright's Disease, *J. Clin. Investigation* 13: 969, 1934.

5. Freyberg, R. H.: The Choice and Interpretation of Tests of Renal Efficiency, *J. A. M. A.* 105: 1575, 1935.
6. Stieglitz, E. J., and Knight, A. A.: Sodium Ferrocyanide as a Clinical Test for Glomerular Efficiency: Preliminary Report, *J. A. M. A.* 103: 1760, 1934.
7. Shevky, M. C., and Stafford, D. D.: A Clinical Method for the Estimation of Protein in Urine and Other Body Fluids, *Arch. Int. Med.* 32: 222, 1923.
8. Rowntree, L. G., and Geraghty, J. T.: The Phthalein Test: An Experimental and Clinical Study of Phenolsulphonephthalein in Relation to Renal Function in Health and Disease, *Arch. Int. Med.* 9: 284, 1912.
9. Christian, H. A.: The Use of Tests of Renal Function in Cases of Nephritis, *J. Urol.* 1: 319, 1917.
10. Mosenthal, H. O., and Bruger, M.: The Urea Ratio (Urea Nitrogen Per Cent of Total Nonprotein Nitrogen in the Blood) as a Measure of Renal Function, *Trans. Assoc. Am. Phys.* 49: 82, 1934.
11. Moeller, E., McIntosh, J. E., and Van Slyke, D. D.: Studies of Urea Excretion. II. Relationship Between Urine Volume and the Rate of Urea Excretion by Normal Adults. III. The Influence of Body Size on Urea Output, *J. Clin. Investigation* 6: 427, and 467, 1928.

G. H.

Correspondence

Dear Ed.:

Have you ever noticed that the simplest little discovery may have the most important results? Sir Isaac, for example; if he had seen the apple fall up instead of down we would never have had the Law of Gravity.

I have recently made a simple discovery that may be quite important. I have found that I can nail three medical magazines together as well as three boards.

In our community we have some medical colleagues who would not dream of committing a criminal abortion or of splitting a fee, even by letting the family doctor assist at the operation. But these same honorable gentlemen, when it comes to a loose issue of, say, the *JOURNAL OF LABORATORY AND CLINICAL MEDICINE*, which contains an article that interests them, display a capacity for the differential diagnosis of mine and thine that is quite lousy.

For many years and particularly during the late (?) depression, when expenditures for bookbinding were out of the question, it was my habit, at quarterly intervals, to bore holes in each set of three journals, string them together with picture wire and solder it in place. We lost fewer journals but it was a monumental task to bind some 45 different journals and the picture wire was rough on the table tops.

Now I nail the journals together. It sounds so simple that it sounds silly. I line three journals up at the edge of a table, turn back the cover, clamp them to the table with a pair of carpenter's clamps for better immobility, and with three wire nails with heads, of a length that will not quite permit of complete penetration, nail them together. Then I turn the journals face down, turn back the back and drive three more nails. Headed wire nails, 1¼, 1, and ¾ inch are best suited. An honest doctor may steal one journal but three together wouldn't go into an overcoat pocket, even if we wore overcoats in Hawaii.

You probably will not publish this letter for I see that you are in the bookbinding business.

At the risk of seeming just too erudite, I add another discovery, simple but with far-reaching consequences; I have discovered that since holding the nail I was driving with a pair of forceps, I haven't hit my thumb once.

Aloha,

E. A. FENNEL.

Honolulu.

February 3, 1936.

The Journal of Laboratory and Clinical Medicine

VOL 21

JULY, 1936

No 10

CLINICAL AND EXPERIMENTAL

EXPERIMENTAL AND CLINICAL OBSERVATIONS REGARDING ANGINA PECTORIS AND SOME RELATED SYMPTOMS*

D E JACKSON, PH D, M D, AND HELEN L JACKSON, M D,
CINCINNATI, OHIO

IN OUR opinion angina pectoris is due to acute, spasmodic, incoordinated contractions of the esophagus and stomach. By this reaction an or other stomach contents are entrapped either in the esophagus or in one portion of the stomach. That such action occurs in the human subject is well demonstrated by the fact that more than forty cases have now been described in the literature in which at autopsy complete rupture of the esophagus has been found. The symptoms in these cases generally resemble those of coronary thrombosis as now usually recognized, and confusion and mistakes in the diagnosis are easily understood. Perhaps the most striking case of rupture of the esophagus was that described (the first on record) by Hermann Boerhaave¹ in the case of Baron de Wassenaer in 1724. In this case the esophagus (so nearly as we can determine) was torn completely in two a little ways above the diaphragm. This indicated an endwise pull (by vomiting in that case) on the esophagus, but the usual rupture is a longitudinal slit, generally on the left side about one inch above the cardiac sphincter. While more than forty of these cases are proved to have undergone *complete* rupture, we have found but little emphasis placed in the literature on what we believe to be a vastly greater number of cases in which partial rupture, or temporary straining, occurred. This is the type of case (the stomach may be included) which we believe represents what is now known as angina pectoris. If there be no rupture either into the lumen of the esophagus or into the pleural cavity, then no visible hemorrhage may be detected even at autopsy. But it was well

*From the Department of Pharmacology of the University of Cincinnati College of Medicine

known even to Heberden that these patients occasionally vomit and that sometimes the vomitus contains blood. The presence of ulcers or other lesions in the esophagus, gallbladder or stomach does not change the viewpoint, but, only makes more probable the occurrence of incoordination and spasticity through the local nervous mechanism. When pain radiates down one or both arms, then we believe that it originates in that portion of the esophagus and adjacent (strained or injured) mediastinum which lies between the horizontal midline of the heart and the upper opening of the thorax. The evidence for this will appear below. But if the pain remains in the neighborhood

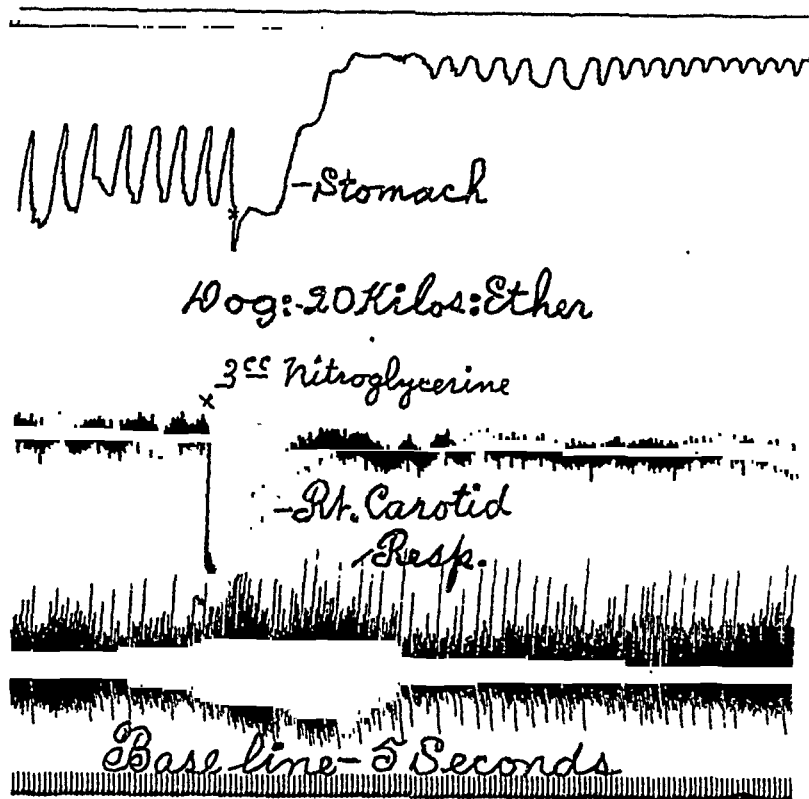


Fig. 1.—Upper tracing, stomach; middle tracing, blood pressure; lower tracing, respiration. At "X" 3 c.c. of nitroglycerine (½ per cent) were injected by vein. Stomach contractions immediately ceased and the walls relaxed. This permitted the longitudinal fibers, to which the instrument was attached in front of the greater curvature, to shorten passively. This caused the writing lever to rise, but the stomach as a whole was completely relaxed.

of the ensiform or a little way above and does not radiate laterally (to arms, etc.), although it may extend up into the neck, jaws, and those parts of the face and neck which are innervated by the tenth nerve and the fifth nerve (which has communications with the tenth), then we believe that the origin of this type of pain (probably chiefly vagus) lies mainly in the stomach, or in the stomach and the lower end of the esophagus.

Perhaps not a single extensive article on angina pectoris from Heberden on down has failed to note that the relief of pain in this type of case is often

associated with the eructation of a large volume of gas. Our own observations, both experimental and clinical, lead us to suspect that the relief of the pain really begins a little while before the gas escapes. This is due, in our opinion, to the beginning relaxation of the stomach wall and of the cardiac sphincter and perhaps of contracted segments of the esophagus (in which the longitudinal muscle layer is involved). This permits the gas to escape. That the relaxing action of nitrites on smooth muscle may greatly accelerate this process will be obvious at once to all pharmacologists (Fig 1). No gas or other stomach contents may escape through the mouth, however, for relaxation of the stomach may permit their painless retention. (This, we believe, has actually been seen fluoroscopically in man by Payne and Poulton.)

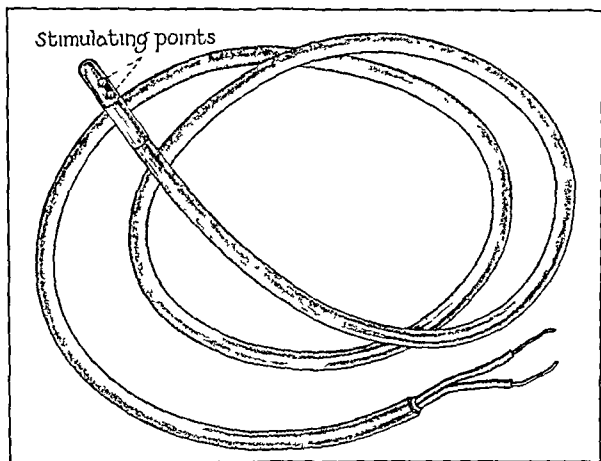


Fig 2—Special electrodes for intraesophageal stimulation. For description see text.

Fig 2 shows a new form of stimulating electrodes made of two parallel wires insulated with a rubber covering about three eighths of an inch in diameter. The wires, including the covering, are about as flexible as an ordinary stomach tube and are four and one half feet long. At the stimulating end (hard rubber tip, water tight connections) two small screws make contact with the inside wires. The flattened heads of these screws form the stimulating points of the electrodes. When the tip of these electrodes is passed down into the esophagus of an anesthetized dog and a stimulating current is applied, a variety of results may be obtained. If the electrodes are placed a little way above the cardia stimulation may inhibit breathing. If the electrodes be moved a little way other nerve fibers may be caught which will cause vigorous and very rapid respiratory movements. Mixtures of these two effects may

also be obtained. Apparently they are due to reflex stimulation of the respiratory center. As the electrodes are drawn upward in the esophagus and new areas are stimulated, other phenomena occur, one of these being either slowing of the heart (possibly vagus inhibition), or the sudden development of auricular fibrillation. In most, but not all, dogs the auricular fibrillation can be produced readily. In some animals the fibrillation stops when the current is turned off, but in others it may persist for some minutes (Fig. 3). The type of anesthetic used undoubtedly influences the results. This is a good

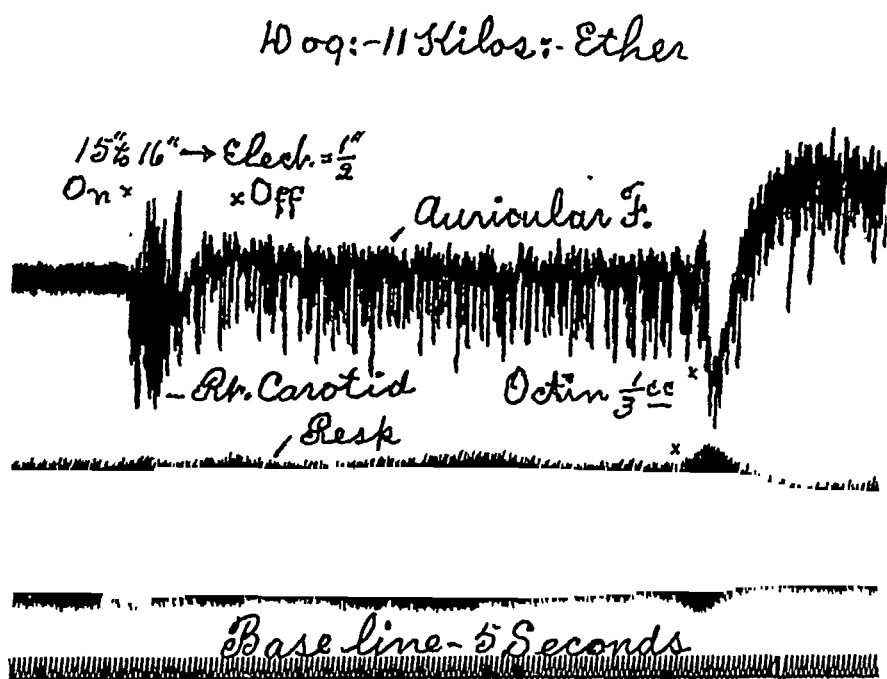


Fig. 3.—Upper tracing, blood pressure (carotid artery, mercury manometer). At "on" electrical stimulation was started within the esophagus directly behind the left auricle. The auricle promptly started to fibrillate and continued to do so throughout the rest of the tracing although the stimulation was discontinued at "off." Near the end of the tracing an injection of "octin" first lowered and then raised the blood pressure but did not stop fibrillation. Later an injection of adrenalin restored the normal beat.

way to produce auricular fibrillation for students who wish to study the phenomena in the intact animal. We believe that fibrillation is produced by leakage of current through the esophagus wall into the left auricle, but the sympathetic nerves in that area may be somewhat involved (as may some vagus fibers). We have not ventured to try this experiment in man for fear of the development of dangerous cardiac irregularities.

Stimulation within the lumen of the esophagus at most points above the level of the apex of the heart will produce vigorous muscular movements in some area of the chest, or in the fore limbs, neck, abdomen, or other parts of the body. This is due to stimulation within the esophagus of nerves which, in some way, transmit the impulses to the muscles of the involved areas. In the nonanesthetized dog weak stimulation thus applied moves exactly the same muscles (and apparently finer divisions of the musculature) and at the same time produces unmistakable symptoms of pain. The innervation appears to be *strictly unilateral* (and we believe this point to be of special importance particularly with reference to the injection treatment of angina). When the stimulating points are turned to the right, then movements (and pain if the animal is conscious) are produced in the right side. If the points are turned to the left, the movements (and pain) are on the left. But if the stimulation is toward the front or the back of the esophageal lumen, then movements may be obtained on both sides apparently because the current spreads a little toward each side. With a little care one can pick out exactly the area into which pain spreads in angina pectoris (left arm, right arm, chest, etc.). We should emphasize here that these effects as seen in the *anesthetized dog* are mainly motor. But we have obtained a description of the muscular pains and soreness down the left arm of a patient only a few hours after he had had a severe attack of coronary thrombosis which involved, so far as it is possible for us to make comparisons between the human being and the dog, exactly the same muscles.

We should emphasize that by simply rotating the stimulating points from the left to the right within the esophagus we can produce exactly comparable movements on the right side. We believe that none of the involved nerves come from the true muscular tissues of the heart nor do they have anything to do with the heart directly (but the heart may be involved indirectly, or by reflex nervous action). For the strength of the heartbeat, the blood pressure, etc., may be entirely uninfluenced by the stimulation unless an area just beneath (behind) the auricle be stimulated (and then no movements of the limbs may occur). But if a few (efferent) sympathetic fibers going to the heart are caught in the stimulation, or if extra adrenalin is secreted, then there may be slight (delayed) changes in heart rate, etc.

Clinically it is well known that soreness of certain groups of muscles (usually down one arm) may follow attacks of angina pectoris or coronary thrombosis. Very rarely a herpes zoster follows down the course of the nerves in the arm and more often swelling, redness, other skin lesions or weakness occurs. We believe all of these symptoms result (directly or indirectly) from injury of these nerves in the esophagus wall or in the neighboring mediastinum and pleura. There is a good deal of clinical evidence to indicate that adhesions (from infections etc.) between the esophagus and adjoining structures (pleura, bronchus etc.) may be concerned in some cases. In such instances especially the longitudinal fibers of the esophagus (many muscular strands from the esophagus connect with neighboring structures) may produce a pull on nerve fibers which would be painful. Such endwise contrac-

tions of the esophagus can be seen if the outer wall of the organ is stimulated. These movements are of a rapid "creeping" character when produced by faradization. The esophagus seems to be capable of shifting its own position (also the effects of stimulation within it) somewhat from side to side (at least in the dog).

The difficulty of explaining both the presence and the absence of pain in the heart (coronary arteries?) during attacks of angina or coronary thrombosis has impressed many observers. And many literary (but fewer experimental) attempts have been made to clear the matter up. Within recent years von Bergmann,² Gionnoni and Lunedei,³ and others have advanced the view that stimuli passing up the vagi from the stomach or esophagus go to the medulla and are thence reflexly sent back down over other vagus fibers (not having yet been felt by the patient) to the heart where constriction of the coronary vessels is produced, and this causes painful impulses which pass back through the posterior (anemia nerve?) roots to the spinal cord and thence to the brain when the patient becomes aware of their presence. We believe this conception is mainly erroneous, but partly correct. Von Bergmann has well described a whole series of patients (with x-rays) showing that "hiatus hernia" or dilatation of the esophagus at or just above the cardia is frequently found in patients having angina or "anginoid" symptoms. He has seen such a hernia which would balloon up suddenly just above the diaphragm when the patient breathed deeply. Many of these hernias are developed suddenly during fits of vomiting or straining. One of our patients dated her trouble definitely from the "breaking loose of something" deep behind the ensiform while she was attempting to place a heavy box on a high shelf. She died suddenly about four years later after having run a typical course of angina pectoris. We have found no x-ray pictures in the literature showing the condition of the stomach and esophagus (*with contained barium*) during an acute attack of angina pectoris or coronary thrombosis. But we know that vomiting is nearly always present in acute coronary thrombosis and sometimes in anginal attacks. The eructation of gas, or its retention in a relaxing (or contracting) stomach, of course, would not be seen by the patient or his attendants. Von Bergmann has expressed the opinion that there occurs an endwise (up and down) pull by the esophagus on the stomach, so that folds of the stomach may be drawn up through the esophageal orifice in the diaphragm (as shown in his x-ray pictures). This may be partly due to descent of the diaphragm in deep breathing which tends to compress the stomach.

"Hiatus hernias," diverticuli, dilatations, spasms and other anomalies or lesions of the esophagus in great number and variety are, of course, well known to gastroenterologists, but there is extremely little emphasis placed on these by cardiologists. And yet if one can get a true history of the exact location of the very first sensation which a patient feels in an attack of angina or coronary thrombosis, and if the physician can imagine that such an instrument as an ice pick were passed backward through the initial point of pain to the posterior wall of the body, it will very generally be found that the

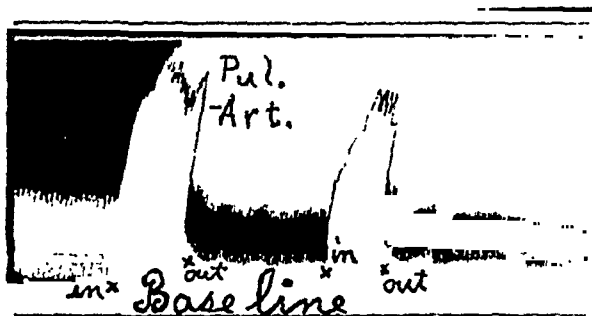
instrument would pass through the esophagus or the upper part of the stomach. But secondary radiating pain in other regions may often be so severe that its point of origin is lost sight of entirely.

Especial mention should be made of the work of Walter Verdon⁴ in England. Verdon believed that the real lesion of angina pectoris (he did not seem to recognize coronary thrombosis) consisted in the establishment of "mutable centers" in the spinal cord or medulla. He believed these "centers" were excited by stimuli that originated in the walls of the stomach or esophagus from pressure inside the organs which produced stretching (tension) on the gastric or esophageal outer coats, and that when these impulses reached the "mutable centers" the impulses were reflected outward over somatic nerves to the left arm, shoulder, neck, or other regions to which the pain was referred. A very large number of valuable clinical observations were made by Verdon. He did no animal experiments and does not mention the electrocardiograph, nor coronary thrombosis as we recognize it today.

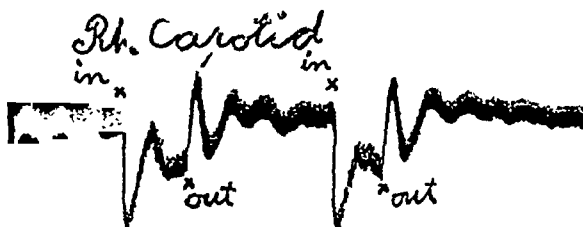
If one opens the chest under anesthesia and records the pulmonary blood pressure from the left pulmonary artery, it will be found that inflation of a rubber balloon in the esophagus just behind the heart will cause an enormous rise in the pulmonary blood pressure (Fig. 4). A similar inflation of the esophagus by entrapped air (or stomach contents), due to two approaching spasmodic contraction rings, may very well be expected to produce a comparable physiologic effect with increase of pressure in the right heart (causing, as can be shown experimentally, axis deviation, T wave changes, etc.), dyspnea, dysphagia or anginal pain. It has been fully demonstrated by Hertz and by Payne and Poulton⁶ and by others that rapid inflation of a balloon in the esophagus (even with low pressures) causes the development of pain which may easily be made so acute that the subject cannot stand it, and that the subject can locate the position of the pain (bag), as revealed by fluoroscopy, within one inch of its exact location. It is our opinion that a patient who indicates over the left border of his sternum, or over the ensiform a spot which marks the seat of his pain deep down in the chest is actually locating within one inch the exact origin of his pain in the esophagus or neighboring tissues. If the pain radiates down one or both arms or into the chest or back then we believe that the esophageal dilatation (or endwise traction) has involved the nerves (in the esophageal wall, mediastinum, pericardium or pleura) which we have discussed above with reference to intraesophageal electrical stimulation.

When a bag is inflated in the esophagus behind the heart the presence of the bag may impede or close off passage of blood through the pulmonary veins from the lungs back to the left auricle. This may tend to lower the aortic pressure but to cause engorgement (possibly painful) and cyanosis on the systemic venous side. The position of the bag (or dilatation) up or down the esophagus will vary the results obtained. We believe that this phenomenon is most likely the cause of so called "cardiac asthma" (Fig. 4). But there are at least two other possibilities which we have considered. These are the possibility that the dilatation may mechanically compress the bronchi (or

auricle), or that dilatation (possibly longitudinal contraction) of the esophagus may stimulate respiratory-inhibitory nerves in the esophagus as we have above indicated (by electrical stimulation in our experiments). A cross-section of the chest at the level of the base of the heart will show that the esophagus lies directly behind the heart and that the pulmonary veins (posterior—low or negative pressure) are most likely to be compressed before the pulmonary arteries are affected. Incidentally, in passing, it may be noted that a pain in the base of the heart or one in the adjacent esophagus might be only about a quarter



Sod. Amytal-27grs.



Bag Inflated in Oes.

Dog:-16 Kilos:-

Baseline-5 Seconds.

Fig. 4.—Upper tracing, pulmonary blood pressure; lower tracing, carotid blood pressure. Inflation of a rubber balloon (twice) in the esophagus behind the heart (compressing the pulmonary veins and left auricle) blocked the return flow of blood from the lungs to the heart and greatly raised the pulmonary pressure but tended to lower the systemic pressure. For discussion see text.

of an inch apart, and it would be quite impossible for the patient, let alone the heart specialist, to distinguish between these locations on the basis of the pain alone. And any sensory nerves coming from the heart would pass through this region, and hence would be subject to injury at this point.

Experimentally in dogs and fluoroscopically in man, it can be shown that the esophagus may frequently undergo peristaltic movements both upward and

downward (Fig 5), especially during vomiting, retching, and similar states. For this experiment it is advisable to fill the esophageal bag with water rather than air. The local nervous mechanism⁷ seems capable of carrying on these movements independently of the vagi and sympathetics, but no doubt these nerves always exercise a control under normal conditions. But under strong emotional stress vagus action undoubtedly can influence these peristaltic movements both in the esophagus and in the stomach. Thus we believe to be the chief etiology of "nervous indigestion" and of emotional anginal attacks.

A full stomach, or one containing *much gas*, we believe to be the usual initiating cause of an acute attack of angina pectoris, or of the commonly

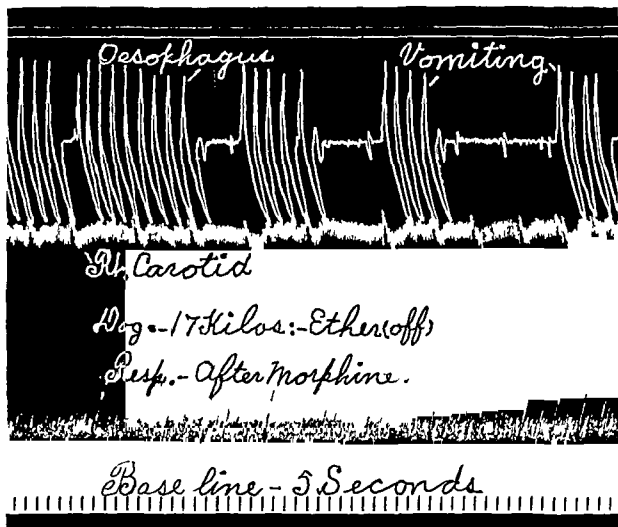


Fig 5—Upper tracing esophagus middle tracing blood pressure lower tracing respiration. Esophagus tracing obtained by placing a rubber bag filled with water in the esophagus just behind the heart. Vomiting, retching, nausea, etc. produce slowly moving peristaltic contractions in the esophagus. In man similar movements are carried out and the subject is often entirely unconscious of them. We believe such movements as these (exaggerated) and also longitudinal movements pull on nerves or other structures in the mediastinum to produce the pain of angina (and coronary thrombosis).

recognized form of coronary thrombosis. A high strung, nervous individual whose stomach becomes upset following a sudden death in the family (we now have two such patients under observation), or following any other incident which greatly excites his emotions, is liable to be seized by an anginal, or "anginoid" attack (perhaps the first he has ever experienced). We believe all of these attacks to be of essentially similar character and etiology but to vary in degree and in the location of the pain (which often is not pain but rather dyspnea, see Fig 4). These results may be brought about in at least

three different ways. First, exercise leads to increased breathing and hence increased compression of the stomach by the diaphragm and abdominal muscles. Von Bergmann's x-ray plate shows a patient with a hiatus hernia into the esophagus which could be seen to fill up suddenly when a deep breath was taken. The presence of such foreign material in the esophagus, and under increasing pressure from the stomach, will be very likely to stir up spasmodic, incoordinated contractions in the esophagus. In stimulating the lumen of the lower end of the esophagus in nonanesthetized dogs with a weak current, we have usually produced vomiting, among other phenomena. And vomiting in dogs is associated with irregular, peristaltic movements of the esophagus (see Fig. 5). And Hertz, and Payne and Poulton have shown clearly that pressure (especially sudden) inside the esophagus produces pain which increases in intensity with the pressure, and which the patient can accurately locate. We cannot be sure but that the longitudinal muscle layers of the esophagus (especially in the presence of pleural adhesions) may cause some of these results. And a further point to be emphasized is the increased intermittent pulling on the lower end of the esophagus (smooth muscle tonically resists tension) which deep breathing from exercise may bring about, especially with a full stomach.

Second, we believe an "overflow" of nervous impulses along the vagi to the stomach and esophagus may stir up abnormal contractions, and in a susceptible patient these contractions may become incoordinated and thus entrap air or other stomach contents in the stomach or esophagus or both. This is the "emotional" type of attack (sometimes cured by psychotherapy), and it is directly related to "nervous indigestion" of which the patient may or may not complain. It must be dependent on anatomical abnormalities in the stomach or esophagus (or adhesions of the esophagus), or to derangement of the local nervous mechanism in the walls of the esophagus or stomach, or to a central derangement of the impulses which pass down the vagus (possibly the sympathetics) to the esophagus and stomach. The stomach wall contains only smooth muscle while the lower third of the esophagus is smooth muscle, the middle third mixed, and the upper third is entirely striated. But the entire length of the esophagus is innervated (vagi and sympathetics) in exactly the same fashion as an ordinary smooth muscle structure. Hence, we would not be surprised to find occasional anomalies in esophageal contractions, especially in the middle third, and these would be likely to extend to other parts of the esophagus. We have collected a good deal of data which make us suspect that some individuals have a fair amount of voluntary (but unrecognized) control over certain esophageal contractions. For example, we had a patient who could "fight off" anginal attacks quite often. We believe that was done through the vagi by some form of inhibition, or recoordination, of esophageal or stomach contractions. There are other such cases indicated in the literature. (We consider here, of course, that the coronary arteries have nothing whatever to do with angina, but angina may often secondarily involve the heart.)

Third, attacks of angina may be induced directly by indigestion. These are more likely than the other types to lead to acute coronary thrombosis and frequently are fatal, either within a day, or within two to four weeks. But

partial recovery with later attacks may frequently occur. These patients may be hale and hearty, are usually good eaters, often fan drinkers, and may or may not have previously had "indigestion." The Cincinnati papers usually carry notices of four or five of these cases every day. Attacks may come on at any time, often in sleep. If the patient makes a good recovery with no observable lingering after effects, he is said to have had indigestion (Fig. 6)

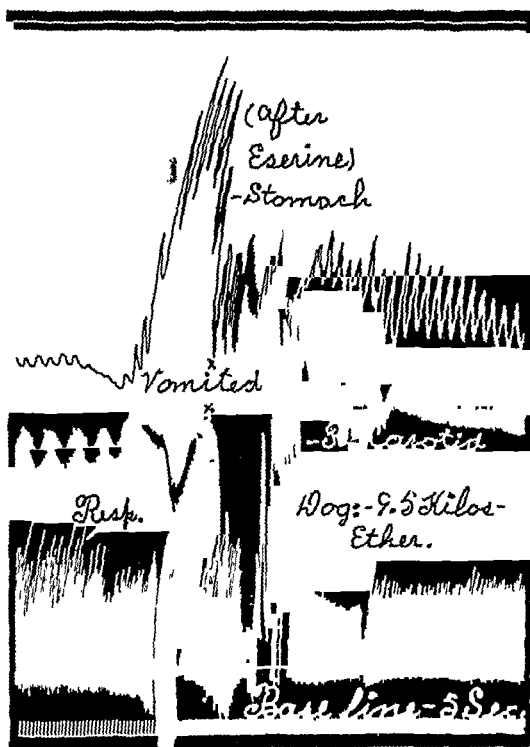


Fig. 6—Upper tracing stomach middle tracing blood pressure lower tracing respiration. A very small amount of eserine had been given to partly overcome the ether depression so that stomach contractions could be obtained. Following a large stomach contraction the animal vomited at X. There was some respiratory difficulty and then the heart was strongly inhibited (reflexly) and finally stopped for half a minute or more. The heart finally broke through the inhibition and returned to normal. Such excessive inhibition is apparently the cause of death in many cases of angina pectoris.

But if he gets a severe painful esophageal or mediastinal injury with edema around the roots of the lungs, slight fever, some leucocytosis, perhaps with distant heart sounds and a pericardial friction rub (probably from dis-

placement of the heart by mediastinal swelling), possibly electrocardiographic changes or fall in blood pressure, or, especially if he dies within a few hours, then the diagnosis is coronary thrombosis. But if he dies suddenly in the early stages of the attack, then it is angina pectoris (see Fig. 6). At the autopsy the coronaries may be found to be entirely normal with little or no visible pathology in the heart. Or an actual thrombosis may be present. We believe that the pain in these cases is not due to the coronary thrombosis *per se*, but to esophageal or mediastinal injury (the stomach may be involved in some cases, i.e., in the region of the cardia). The pain from an actual rupture of the tissues with absorption, etc., will, of course, be likely to last for at least some hours.

Why sudden death in these cases? Fig. 6 illustrates well what is probably the most common cause, i.e., excessive vagus inhibition from reflex stimulation of (sensory) vagus fibers by stretching or injury of the esophageal wall (the stomach may be equally involved) in the act of vomiting or by entrapment of gas (without vomiting). When no thrombi are present in the coronaries, i.e., when the chief initiating cause of death lies *outside* the heart, we believe from both clinical and experimental evidence that inhibition is the most probable cause of death. But when the coronaries are thrombosed (i.e., the chief initiating cause of death may be *within* the heart), then we suspect that ventricular fibrillation will be the most likely cause of sudden exitus. Slow death from weakening of the heart and general exhaustion may, of course, occur in these cases.

It is interesting to note that the first case of coronary occlusion ever diagnosed during life (by Adam Hammer⁸ in St. Louis in 1876) occurred in a patient who had *no pain whatever* during his attack, which lasted some thirty hours, and was due to the formation on an aortic valve of a thrombus which extended up and occluded the right coronary artery. The total absence of pain in this case (and there are many others in the literature now, Herrick,⁹ Davis,¹⁰ Levine¹¹) convinces us that anemia of the heart *per se* (from constriction of the coronaries or anything else) does not produce the pain of angina pectoris. And there is an abundance of other evidence, both experimental and clinical, to prove this point, which (together with other vague notions of pain in the *heart*) has hampered medical progress with respect to angina pectoris ever since the suggestion involving the coronary arteries was first surmised by Jenner. And the suggestion that angina pectoris was like intermittent claudication, in our opinion, only made bad matters worse.

Drugs.—Nitrites we believe exercise their beneficial action by relaxing the stomach and esophagus. We have personally felt a relaxation of the pyloric end of the stomach by nitroglycerine which promptly relieved colicky pains which we knew to be due to gas in the stomach. This is obviously their action in seasickness. Theophylline and its congeners may afford some relief by their diuretic action, but there is some evidence that they may exercise a very slight sympathetic (adrenalin-like) action, and we believe that such slight benefit as they may afford is due to this action on the stomach and esophagus. Digitalis irritates the stomach and is liable to make angina cases worse, but the

drug may be indicated for coincident cardiac conditions. Morphine may relieve the pain of angina pectoris not only by its central action but also by relaxing¹² the stomach walls. Papaverine acts similarly on the stomach (not centrally). Atropine relaxes the stomach, but the required dose is probably too large to be of use clinically. Adrenalin relaxes the stomach and (to a less extent) the esophagus, but its central and other actions (fear, excitement) are liable to induce attacks. It dilates the coronary arteries¹³. Administration by mouth (slow and imperfect absorption) may be helpful in certain cases. Trichlorethylene has been used by Kiantz. It acts centrally as an anesthetic. Insulin, by indirectly increasing the (hunger) contractions of the stomach may make the attacks worse.

Within recent years much brilliant surgical work has been done on angina pectoris. In our opinion the chief conclusion to be drawn from all this work is that it has added further evidence to show that the coronary artery explanation of angina pectoris is wrong, and that the explanation we have offered above is correct.

Finally, it has been shown by Verdon¹⁴ "on many occasions and always with satisfactory results" that during an acute attack of angina pectoris passage of a stomach tube through the esophagus into the stomach has led to prompt elimination of a large volume of gas and sometimes of fluid contents of the stomach, and to the immediate, usually complete, relief of the patient. We believe that practically every conclusion regarding this disease that has heretofore been reached on a basis primarily of pathology alone is wrong. For, in our opinion, angina pectoris is an affliction of the living only, not a state of the dead.

CONCLUSIONS

1 We have shown that electrical stimulation inside the esophagus at appropriate locations within the chest produces muscular contractions and pain in exactly those areas of the body in which pain (and later soreness) is developed during acute attacks of angina pectoris and coronary thrombosis. The innervation we believe is strictly ipsilateral and does not come from the heart.

2 We believe that the coronary artery spasm explanation of angina pectoris is erroneous.

3 We believe that angina pectoris is due to acute incoordinated spasmodic contractions of the esophagus (including its longitudinal muscle layers) and stomach whereby gas or other stomach contents are entrapped under pressure and the walls of either viscous with their contained or adjacent nerves and tissues are strained or injured. We have presented a small part of our extensive experimental and clinical evidence to support these views, and we have referred briefly to coronary thrombosis.

REFERENCES

- 1 Boerhaave, Hermann. *Morbi Historia Lugdunum Batavorum*, 1724.
- 2 Von Bergmann, G. *Das Epiphrenale Syndrom*, "seine Beziehung zur Angina Pectoris und zum Kardiospasmus." *Deutsche med. Wochenschr.* No. 16, 605, 1932.
- 3 Giannoni, Alberto, and Lunedei, Antonio. *Tentativo di riproduzione sperimentale nell' uomo della sindrome epifrenica e della angina pectoris d'origine gastrica*, *Riv. di clin. Med.* 35, 569, 1934.

4. Verdon, Walter: *Angina Pectoris*, Baillière, London, 1920, Tindall and Cox.
5. Hertz, Arthur F.: *The Goulstonian Lectures on the Sensibility of the Alimentary Canal*, Henry Frowde, London, 1911, Oxford University Press.
6. Payne, W. W., and Poulton, E. P.: Visceral Pain in the Upper Alimentary Tract, *Quart. J. Med.* 17: 53, 1923; Pressure and Stretching as Independent Factors in the Production of Esophageal Pain, *J. Physiol.* 56: 53, 1922; Experiments on Visceral Sensation, Part I. The Relation of Pain to Activity in the Human Esophagus, *J. Physiol.* 63: 217, 1927; Part II. The Sensation of "Nausea" and "Sinking"; Esophageal Reflexes and Counter-Irritation, *J. Physiol.* 65: 11, 1928.
7. Burget, G. E., and Zeller, W. E.: Observations on the Cardia in Unanesthetized Animals, *Am. J. Physiol.* 116: 21, 1936.
8. Hammer, Adam: A Case of Thrombotic Occlusion of One of the Arteries of the Heart, *Wien. med. Wchnschr.* 28: 102, 1878. Quoted from Major, Ralph H.: *Classic Descriptions of Diseases*, Baltimore, 1932, Thomas, p. 397.
9. Herrick, James B.: Clinical Features of Sudden Obstruction of the Coronary Arteries, *J. A. M. A.* 59: 2015, 1912; *Ann. Int. Med.* 4: 105, 1929; *Am. Heart J.* 6: 589, 1931.
10. Davis, Nathan Smith, III: Coronary Thrombosis Without Pain: Its Incidence and Pathology, *J. A. M. A.* 98: 1806, 1932.
11. Levine, Samuel A.: Coronary Thrombosis, Its Various Clinical Features, Baltimore, 1931, The Williams and Wilkins Co., pp. 23-113.
12. Plant, O. H., and Miller, G. H.: Morphine Constipation, *J. Pharmacol. & Exper. Therap.* 32: 437, 1928.
13. Mann, F. C.: Personal Communication; and others.
14. Verdon, Walter: *Loc. cit.*, p. 287.

COMPLETE TEMPORARY RECOVERY, OF LONG DURATION, IN ACUTE ALEUCEMIC MYELOID LEUCEMIA*

A CASE REPORT

ISRAEL H. MARCUS, M.D., BROOKLYN, N. Y.

RECOVERY, even temporary, with *complete* return to normal of the symptomatology, physical signs, and especially the blood picture, is so rare in myeloid leucemia, that the following case is reported in detail.

C. N., a Hebrew housewife, fifty-eight years of age, entered the hospital on May 5, 1930, having suffered for nine weeks with anorexia, lassitude, increasing pallor, fever of 100.5° to 103.5° F., pain and swelling of the knees, elbows, ankles, and wrists, the joint symptoms persisting for a day or two in one joint and then traveling to another. During the last four weeks there had appeared at separate occasions on the left forearm two vesicles, containing serosanguineous fluid, upon ecchymotic bases and surrounded by induration and erythema. Each had persisted for about a week.

Family History.—One sister has Hodgkin's and one brother has Vaquez's disease.

Previous History.—Unimportant except for pneumonia in 1918.

Physical Examination on Admission.—Temperature 103° F. Marked pallor of both skin and mucous membranes. *Mouth:* The tonsils were buried and cryptic, and much foul smelling discharge could be expressed from the left one. *Chest:* No sternal tenderness. *Lungs:* There was dullness, decreased breath sounds, and a few large moist râles at both bases. *Heart:* Slightly enlarged to left. There was a soft systolic apical blow. *A₂* was louder than *P₂*. Blood pressure 130/80. *Abdomen:* Liver edge was felt 2 fingerbreadths below

*From the Meyer A. Rabinowitz Medical Service of the Jewish Hospital of Brooklyn.
Received for publication, July 9, 1935.

the costal margin. Tip of spleen was barely felt. *Extremities* No edema. The knee joints, elbows and left wrist were painful on active and passive motion and were tender. Both knee joints were swollen. No redness of the joints.

Laboratory Findings—Blood Wassermann and Kahn tests, blood cultures, blood Widal, and blood chemistry examinations revealed no abnormalities.

X Ray of the Chest on Admission—Negative.

Progress of the Patient—Shortly after admission a blood study showed 2,000,000 PBC with 36 per cent hemoglobin and 490 WBC with 14 per cent neutrophilic polys of which 9 were band forms and 4 segmented, 40 per cent lymphocytes, 24 per cent myeloblasts and 22 per cent monocytes and only 30,000 platelets. The hematologist believed that the cells he designated monocytes were no true monocytes but were rather forms of myeloblasts. He thought the condition was a true acute myeloblastic leukemia. The blood study, like all the others done on this patient was made by one of a group of physicians who had been specializing in hematology for a number of years. On the day after admission pinpoint hemorrhages were seen in the left conjunctiva and large hemorrhages in both eyebrows. About a week after admission the joint trouble had become markedly ameliorated. Two weeks after admission there was noted a nontender somewhat bluish infiltration in the subcutaneous tissue of the right axilla, not involving the lymph nodes. There were at this time for a few days on the buccal mucosa small spots of whitish exudate, which when rubbed off showed a reddened base. The patient was treated with blood transfusions, liver extract, ventriculin, sodium cacodylate subcutaneously and large doses of iron by mouth. After June 16 the temperature, which had been at 101° to 104° remained at or below 100°. By June 19 the skin infiltration in the axilla had completely disappeared, the liver and spleen could no longer be felt, and the lungs and heart appeared normal. Meanwhile the patient had grown much stronger, and her blood status had markedly improved, though a marked increase in the lymphocytes persisted for a long time. Thus in a study on June 30 she had 4,000,000 RBC and 67 per cent hemoglobin, but there were still 62 per cent lymphocytes. On July 22 a blood study showed 4,300,000 PBC, 54 per cent hemoglobin, 47 per cent lymphocytes, 47 per cent neutrophilic polys with 4 staff forms, 3 per cent eosinophiles, 2 per cent monocytes and 250,000 platelets. The patient was discharged on July 26, 1930.

At home the patient was able to do her usual household duties. On Sept. 10, 1930, a blood study showed 6,000,000 PBC, 52 per cent hemoglobin, 11,200 WBC, with 45 per cent neutrophilic polys, 14 per cent eosinophiles, only 38 per cent lymphocytes, 3½ per cent monocytes, 0.1 per cent reticulation, and 240,000 platelets. No abnormal cells were seen.

On May 9, 1931, i. e., about ten months after her discharge from the hospital, the patient was seen complaining of a cough of two months' duration especially bad for the last few days, anorexia for two months, and weakness for several weeks. Examination showed the patient to have a temperature varying between 100° and 103° each day. She did not appear acutely ill. The pharynx was congested, the tonsils cryptic. Lung examination was negative except for rare rales scattered throughout. The heart examination was negative. Blood pressure 135/90. The edge of the liver and tip of the spleen could now again be barely felt. There was no edema. The superficial lymph glands were not enlarged.

On June 5 many small purpuric spots each 1 to 2 mm in diameter, appeared on the skin of the abdomen and back. The patient was meanwhile growing markedly weaker, and on June 10 she was very dyspneic. Pallor was now marked. On the hard palate and buccal mucosa were several small ecchymotic areas and a small ulcerated area covered with a thick exudate. Her face was full and puffy. At the right lung base there were now heard numerous crepitant rales and diminished breathing. The abdomen was distended and shifting dullness was present. X ray of the chest showed a definite infiltration at the right base with an overlying haze. On June 11 the patient became irrational, the temperature rose to 104° and she died early the next day.

Laboratory Studies—Blood chemistry on admission and two days before death. Normal. Blood culture sterile. On June 10 the urine which had previously been normal, now showed 2 plus albumin, many white blood cells, a rare red blood cell but no casts.

Blood Studies—As is seen in the chart the white blood cells, which on May 14, 1931 were only 8,200 on May 27 were 20,400 and on June 2 34,000. The polys were constantly

but few in number. The lymphocytes at first were as much as 66 per cent, but decreased later to 10 per cent. Many large monocytic cells were seen, which the hematologist, at first not sure whether or not they were atypical myeloblasts, later decided definitely were myeloblasts rather than true monocytes.

COMMENT

It is felt that the findings in this case were so indicative of true myeloid leucemia that the diagnosis was justified even though an autopsy could not be secured. In Naegeli's clinic, too,¹ it is believed that the diagnosis of leucemia can be made on the findings during life in some cases, without postmortem examination.

The difficulty encountered in classifying the large mononuclear cells of this patient's blood as monocytes or myeloblasts illustrates a difficulty frequently met with in acute leucemia.²

The case demonstrates the danger of considering a patient with leucemia as permanently cured until a very long time has elapsed without a recurrence of the disease. Pearce³ reports a cure, although the period of observation after recovery was not four months.

SUMMARY

A case of aleucemic myeloid leucemia is reported, which at the outset appeared to be of an acute type. The patient, however, made a complete recovery for eight to ten months, with no evidence of the disease either on physical examination or blood study, and then again evidenced the findings of acute myeloid leucemia and died.

The case is of further interest because of the following: (1) A family history of having a brother suffering from Vaquez's disease and a sister with Hodgkin's disease, (2) the axillary skin infiltration which later disappeared, (3) the occurrence of vesicles on the skin containing serosanguineous fluid with induration about the vesicles, (4) the onset of the disease with joint manifestations strongly suggesting rheumatic fever, (5) only slight splenic enlargement at any time, and (6) the comparatively large number of lymphocytes found at various stages throughout the disease.

REFERENCES

1. Gloor, W. Ein Fall von geheilter Myeloblastenleukemie, *München med. Wchnsch.* 77: 1096, 1930.
2. Warren, S. L. Acute Leukemia. A Review of the Literature and of Twenty Eight New Cases, *Am. J. M. Sc.* 178: 490, 1929.
3. Pearce, R. M. Acute Lymphocytic Leukemia—Recovery After Intramuscular Injections of Hog Spleen, *Brit. M. J.* 2: 282, 1930.

COMPARISON OF THE XYLOSE TOLERANCE WITH BLOOD UREA IN NEPHRITIC RATS*

HARDY W. LARSON, PH.D., NEW YORK, N. Y.

THE clearance of xylose,¹ used as a measure of renal function, is believed to give results comparable with the urea clearance. Folin² was of the opinion that renal injury should be reflected in a slight retention of urea which would be recognizable, provided the range of normal urea was sufficiently established. He believed that a simple determination of blood nonprotein nitrogen might prove of value in detecting early kidney damage. Peters and Van Slyke,³ however, hold that such a determination is of no value for early diagnosis, and that blood nonprotein nitrogen does not rise until renal impairment is far advanced. The latter view is generally accepted. This study was planned to obtain information on these points.

Because of the extremely low threshold of the kidney for xylose, Fishberg and Friedfeld^{4, 5} advocate the use of this pentose as a delicate index of damaged renal function. They found that after the ingestion of 50 gm. of xylose on a fasting stomach and with limited fluid intake, the blood nonfermentable reducing substances rose to a maximum within three hours, to return to a value approaching the original fasting figure within five hours. If the kidney function was impaired, this five-hour value remained high, and a considerably longer period, depending on the amount of kidney damage, was required before the xylose was removed from the blood stream. In the present investigation animals with progressive nephritis were used to study the points involved.

EXPERIMENTAL

Young rats approximately six weeks old were used for the experiment. One pole of the kidney was ligated to cause degeneration beyond the ligature, and two weeks later the other kidney was removed. The rats were then placed on a diet containing 43 per cent dried liver which was known to produce renal injury. A month later the animals were given xylose. They fasted overnight and were then given 0.1 gm. of the sugar per 100 gm. body weight by medicine dropper, and the nonfermentable sugar of the blood followed for five hours. The rats were bled before xylose was given and one and one-half hours and five hours after the pentose was administered, the blood being obtained from the tail. Two-tenths cubic centimeter of blood was laked in 3.8 c.c. H_2O in a centrifuge tube. One cubic centimeter of a 25 per cent yeast suspension, which had been washed three times, was then added, and the tubes were allowed to stand one-half hour at room temperature. Five cubic centimeters tungstic acid made from equal parts of H_2SO_4 (120 c.c. of 0.666N H_2SO_4 in 1 liter) and

*From the Biochemical Laboratory of the Metropolitan Life Insurance Company, New York.

Received for publication, September 3, 1935.

Presented at the meeting of the American Society of Biological Chemists at Detroit, April, 1935. (J. Biol. Chem. 109: III, 1935).

sodium tungstate (120 cc of 10 per cent $\text{Na}_2\text{WO}_4 \cdot 2\text{H}_2\text{O}$ in 1 liter) were then added, and the tube centrifuged. Five cubic centimeters of the tungstic acid filtrate were used for the nonfermentable sugar determination by the Folin and Malmros⁶ micromethod. Urea nitrogen was determined on 0.2 cc of blood by the manometric method of Van Slyke for whole blood. The urine was collected from the metabolism cages during the fasting period, it was tested for albumin and the sediment examined. This procedure was repeated at monthly intervals until either the xylose curve became abnormal or there was retention of urea. The rats were then killed and the kidneys and livers examined for pathologic changes. Approximately half of the number of kidneys appeared to be normal on histologic examination. The other 50 per cent showed pathologic evidence of early nephritis. The ligature itself did not seem to cause hastening of the process, even in cases where half of the kidney had atrophied. Males were more prone to develop nephritis than females. In the group reported as nephritic, 14 of the 15 were males. In the nonnephritic group, the sexes were about evenly divided. The large majority of rat livers showed albuminous degeneration, and the liver cells appeared swollen.

The results obtained with xylose are very difficult to interpret. The xylose curves are very erratic and show wide discrepancies, and there is no apparent correlation between xylose clearance and urea retention. Approximately half of the rats exhibited xylose clearances which varied considerably from month to month. At times the final value would be back to the fasting level in five hours. The next month it would be high, the following month low, etc. As this test is supposed to be a delicate index of renal function, the conclusion would be that these rats were nephritic one month and normal the next. Certainly there is no evidence of the progressively greater xylose retention from month to month which would be expected if we assume that kidney injury was becoming more pronounced. Some rats showed apparently normal xylose curves for several months before there was any elevation of xylose, although increased values for blood urea and the appearance of casts and large amounts of albumin in the urine indicated much earlier kidney damage. On the whole, the monthly urea values were more consistent than the corresponding xylose figures. Although there were exceptions, the urea values showed less tendency to fluctuate widely, and there was more likely to be a small monthly rise. This is in keeping with the assumed progression of the kidney impairment.

In Fig. 1 the rats are divided into two groups. In the experimental group are those which had one kidney removed and the pole of the other kidney ligated, and which were fed a high protein diet. In this group the rats are classified as nephritic or nonnephritic, according to the pathologic findings. In the other group are the normal or control rats which were not operated upon and which were fed a regular stock diet. The final xylose figure for the fifth hour is plotted as the vertical line. The small circle on each of these lines represents the corresponding urea value for this final determination.

It is seen at a glance that the xylose and urea values do not correspond. There may be high xylose with low urea and vice versa. The average xylose figure for 14 rats showing no evidence of nephritis, and having apparently normal kidneys, is 27.1 as compared with 33.3, the average for 15 rats with

pathologic kidney. It is seen that in the minimum nephritic value and the average for the nonnephritic are approximately the same, as are the nephritic average and the maximum nonnephritic. In the group showing evidence of nephritis on pathologic section, 9 of the 15 xylose values are above the maximum

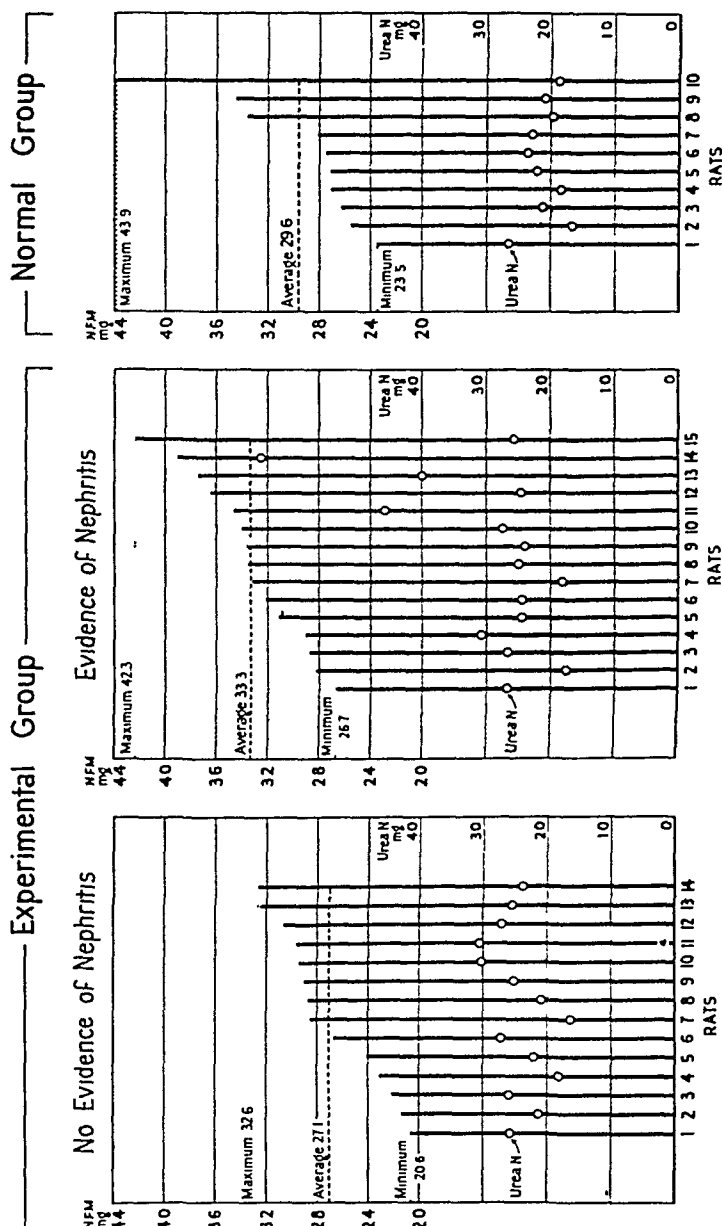


Fig 1—Initial urea nitrogen, and nonfermentable sugar of blood in rats five hours after ingestion of xylose.

for the group showing histologically apparently normal kidneys. This might be interpreted as showing a definite and positive trend toward higher xylose values in rats in which renal injury can be demonstrated by pathologic technic. Ten of the 14 rats in which no histologic evidence of nephritis is found show xylose values lying approximately between the minimum for the group in

which nephritis is shown and the average for this group. Where there is no evidence of histologic change in the kidney, there may still be a functional change not detectable by histologic methods. It may be that xylose clearance and urea retention will indicate renal injury before any histologic change can be demonstrated.

In the nephritic group, Rat 15 shows the highest xylose value, yet pathologically Rats 13 and 14 show a greater degree of renal damage. Likewise, they exhibit much higher urea values, and their urines contain more albumin and a greater number of casts. From this it would appear that they were more nephritic than Rat 15, but the xylose figures do not show it. As the xylose test is assumed to be a delicate indicator of renal function, it would seem that the highest xylose values should be obtained in those rats exhibiting the greatest degree of renal damage pathologically, the most urea and albumin, and which give evidence of functional derangement by the greatest number of casts. Such, however, is not the case.

Although there is a distinct trend toward higher xylose values in the nephritic group as compared with the nonnephritic, the xylose test does not appear as favorably when applied to a group of normal rats. This group of rats was not operated upon and was fed a regular stock diet. They were apparently normal in every respect except in their reaction to the xylose test. Histologically they showed no evidence of nephritis. The urea values were low and the urines contained neither casts nor large amounts of albumin. In spite of this, the xylose figures are not low, as would be expected. They cover a wide range, the average of which is higher than that of the nonnephritic group, and there is a higher xylose value in this normal group than any obtained in the nephritic group. Thirty per cent of the xylose values for this normal group are above the pathologic average. In many cases in this group of normal rats, the results obtained with xylose would lead one to believe that one month some of the rats were definitely nephritic, and the next month normal, etc., because the xylose values were high at times and at other times were back to the fasting level.

Because of the wide variation in the control values, it was felt that this work should be repeated, using a larger number of animals. Twenty young rats of approximately the same age as those used in the control group were given xylose and the blood nonfermentable sugar followed for five hours. Four of this group showed final xylose values which were high. The clearance was repeated at the end of two weeks. Again four final xylose values were high, but only one rat which showed a high xylose in the first clearance was included in this group. The seven rats which gave high values in either the first or second clearance were again given xylose. Six of the seven again showed high xylose values. Only one of the seven gave a high final figure in all three clearances. This again demonstrates the variability of the xylose clearance in normal rats.

The most important finding in this work is the fact that albumin and casts appear in the urine several months before there is retention of urea or the xylose curve becomes abnormal or there is definite histologic change. The presence of albumin and casts indicates a functional disturbance of the kidney which occurs much earlier than the anatomic changes characteristic of nephritis.

In Protocol I, a representative case is taken. The kidney of this rat was reported as showing slight early nephritis. The blood urea remained at a fairly low level for five months, but the albumin increased from 100 to 600 mg. per 100 c.c. within the first month, and the urine sediment showed several casts and cylindroids at the end of the second month. The first xylose curve shows the fifth-hour value approximately down to the fasting normal. The second month shows an elevation. The third, fourth, and fifth months show apparently normal curves. The fifth month shows the fifth-hour xylose value down to the fasting figure at the time 47 granular casts and 40 cylindroids, and an albumin of 800 mg. per 100 c.c. were reported.

PROTOCOL I

EXPERIMENTAL FINDINGS WITH RAT 773 ♂. SLIGHT EARLY NEPHRITIS

DATE	BLOOD		URINE	
	XYLOSE NFM	UREA N	ALBUMIN	SEDIMENT
1933	MG. PER 100 C.C.*	MG. PER 100 C.C.	MG. PER 100 C.C.	
Nov. 16	20.9 39.4 23.4	23.4	100	
Dec. 28			600	
1934				
Jan. 10	18.0 42.8 26.0	26.3	400	2 Granular casts with fat, one with cells. 6 Cylindroids
Feb. 8			700	
Feb. 9	20.9 34.5 23.5	28.3	700	2 Granular casts
Mar. 6	22.9 34.8 26.6	29.4	800	1 Granular cast
April 10	32.2 42.8 32.0	29.1	800	47 Granular casts, all with fat, 10 with cells. 40 Cylindroids
May 8	21.6 45.7 37.4	36.0	700	1 Granular cast with fatty cells
May 9		40.0		

*Initial, one-and-one-half-hour and five-hour values.

History of this rat: Born Sept. 4, 1933. On stock diet Oct. 17, liver diet Oct. 31. Left kidney ligated Oct. 17. Right nephrectomy Oct. 31. Killed May 9, 1934. Body weight 395 gm. Kidney 23 x 18 x 12 mm. Weight 2.15 gm. One-third of kidney absorbed. Marked atrophy beyond ligature. Slight early nephritis.

Protocol II gives data on a representative rat whose kidney showed no histologic evidence of nephritis. Here again there are apparently normal xylose curves, although albumin goes up from 20 to 250 mg. per 100 c.c. and granular casts and cylindroids are reported. The erratic nature of the xylose curves is again shown. Urea values show no progressive degree of retention such as is shown in Protocol I.

The pathologic report on the rat livers is interesting. The large majority have what is commonly called "albuminous degeneration," i.e., the liver cells are full of pinkish staining granules, and they appear swollen. This may be

PROTOCOL II

EXPERIMENTAL FINDINGS WITH RAT 791 ♀ NO EVIDENCE OF NEPHRITIS

DATE	BLOOD		URINE	
	XyLOSE NFM	UREA N	ALBUMIN	SEDIMENT
1933 Nov 22	MC PEP 100 cc *	MG IEP 100 cc	MG PER 100 cc 20	
1934 Jan 5	21.7 29.8 23.8	27.5	250	
Feb 8	18.6 39.8 19.9	21	200	2 Granular casts with fat and cells 3 Cylindroids
Mar 7	21.7 30.2 27.1	25.5	400	2 Cylindroids
April 11	23.7 38.4 23.2	27.0	100	1 Granular cast with fat and cells 1 Cylindroids
May 8	19.3 39.0 32.6	21.9	100	1 Granular cast with fat
May 9		22.9		

*Initial one and one half hour and five hour values

History of this rat: Born Sept 9 1933. On stock diet Oct 23. Liver diet Nov 2. Left kidney ligated Oct 23. Right nephrectomy Nov 2. Killed May 9 1934. Body weight 320 gm. Kidney 24 x 13 x 12 mm. Weight 1.65 gm. One half of kidney absorbed. Slight atrophy beyond ligature. No evidence of nephritis.

due to some toxic effect of the xylose on the liver cells. In this connection, it may be well to report that the rats appeared quite ill after the ingestion of xylose, and diarrhea was common. The xylose test was given to various members of the laboratory staff and the majority reported severe diarrhea.

Fishberg and Friedfeld working with patients manifesting kidney lesions and rabbits with uranium induced nephritis, report that after the ingestion of xylose, the blood curve of nonfermentable reducing substances, instead of approaching the normal fasting value after five hours, continues upward. The writer has performed some 40 xylose clearance tests, using rabbits with uranium induced nephritis. In none of these did the nonfermentable sugar of the blood continue upward after the second hour and practically all fifth hour values approached the fasting normal. In seven instances the fifth hour value was down about 50 per cent from the maximum. In many cases the normal clearance of the animal checked fairly well with the clearances after the animal had reportedly been injected with uranium. Eighteen animals were used in the experiment. Some received as many as six injections of uranium in increasing dosage up to 1 mg per kilo body weight. Histologic examination showed severe kidney lesions.

SUMMARY

The results of these experiments are consistent with the view generally held that kidney function must be seriously disturbed before there is retention of urea. The degree of nephritis produced was not enough to cause marked histologic changes. The values for blood xylose indicate that the use of this sugar as a delicate index of renal function is open to serious question. A more severe

nephritis than that reported here must be produced before consistent decreases in the excretion of xylose can be expected. The excretion of albumin and the presence of casts in the urine still appear to be the earliest indicators of renal disturbance.

The author is indebted to Dr. E. M. Medlar for the pathologic work. He wishes to express his appreciation to Dr. N. R. Blatherwick for helpful criticism, and to Dr. J. M. Connolly and Phoebe Bradshaw for valuable assistance in the work. Pure xylose was generously furnished by the United States Bureau of Standards.

REFERENCES

1. Fishberg, E. H., and Friedfeld, L.: Ausscheidung von Xylose als Massstab der Nierenfunktion, *Klin. Wchnschr.* 12: 218, 1933.
2. Folin, O.: The Determination of Non-Protein Nitrogen in Blood and Its Relation to Nephritis, *Proc. Assn. Life Ins. Med. Dir. Am.* 17: 319, 1930.
3. Peters, J. P., and Van Slyke, D. D.: *Quantitative Clinical Chemistry* 1: 309, Baltimore, 1931.
4. Fishberg, E. H., and Friedfeld, L.: The Excretion of Xylose as an Index of Damaged Renal Function, *J. Clin. Investigation* 11: 501, 1932.
5. Fishberg, E. H., and Friedfeld, L.: Excretion of Xylose as a Measure of Renal Function in Children, *Am. J. Dis. Child.* 45: 271, 1933.
6. Folin, O., and Malmros, H.: An Improved Form of Folin's Micro Method for Blood Sugar Determinations, *J. Biol. Chem.* 83: 115, 1929.
7. Van Slyke, D. D.: Determination of Urea by Gasometric Measurement of the Carbon Dioxide Formed by the Action of Urease, *J. Biol. Chem.* 73: 695, 1927.

COMPARATIVE STUDIES IN CHEMOTAXIS*

JACOB D. KLEIN, M D, CHICAGO, ILL

CHEMOTAXIS is a biologic phenomenon of great significance in immunity and inflammation. There are still controversial questions in the study of this reaction. It is known that various chemical substances may exert a chemotactic influence on leucocytes. Wells¹ mentions the significance of the H ion concentration and states that leucocytes tend to migrate toward the point of greater H ion concentration. Hensheimer,² who studied the effect of acidosis and alkalosis on the blood leucocytes, thought that acidosis increased the cells of the myeloid series while alkalosis increased the cells of the lymphatic series, and ascribed the cause of this response to probable variations in stimulation of the sympathetic and parasympathetic nervous system. In his experiments Hensheimer induced acidosis by inhalation of CO₂, and alkalosis by injection of sodium carbonate until the blood pH rose from 7.32 to 8.52. Gabritschewsky³ (quoted by Wells) studied the chemical influence of a large number of substances on leucocytes and classed them in three groups: (1) Substances exerting "negative chemotaxis" including those that attracted only a few leucocytes, such as concentrated solutions of sodium and potassium salts, lactic acid in all concentrations, (2) substances with "indifferent chemotaxis," which attracted moderate numbers of leucocytes, such as distilled water, dilute solutions of sodium and potassium salts, peptone, glycogen, and bile, (3) substances with marked positive chemotaxis, such as pyrazotim, and sterilized, as well as living cultures of bacteria, whether pathogenic or nonpathogenic. The substances of this group produced general as well as local leucocytosis when injected subcutaneously.

A review of the literature indicates that no law has been determined which accounts for the presence or absence of chemotactic influence in a given substance, since closely related chemical substances may induce entirely opposed chemotactic reactions, while dissimilar chemical substances may display similar properties as concerns chemotaxis.

In the following studies an attempt has been made to compare the local histologic reaction and the general response of the blood, particularly the leucocytes, after the subcutaneous injection of a dilute inorganic acid, a dilute alkali, a dilute organic acid, a typical hormone, and a characteristic bacterial toxin.

EXPERIMENTS

Eight guinea pigs, average weight 300 gm, were prepared by carefully shaving the skin over the lateral part of the abdomen, using the same relative region in each animal. After the area had been carefully washed, and swabbed with mercurochrome solution, the substance to be tested was injected subcutaneously.

*From the John McCormick Institute for Infectious Diseases.
Received for publication September 7, 1935.

At the same time total leucocyte and differential counts were done on each animal. When the experiment was terminated each animal was chloroformed and the tissue carefully excised, fixed in 10 per cent formalin, mounted in paraffin and stained with hematoxylin—eosin, as well as with Wright's stain for special cytologic study. In this way the local tissue reaction was compared with the general reaction as shown by the blood studies. The substances used and compared were dilute HCl (1-1,000), dilute NaOH (1-1,000), lactic acid, 2 per cent hog thyroglobulin, and 1/5 M.L.D. of diphtheria toxin. As indicated in Figs. 1 to 4, guinea pig No. 1 received 1 c.c. of dilute HCl subcutaneously and was killed after twenty-four hours; No. 2 was given 1 c.c. of dilute NaOH and was killed after twenty-four hours; No. 3 received a daily injection of 1 c.c. of dilute HCl for a week; No. 4 received the same amount of dilute NaOH daily

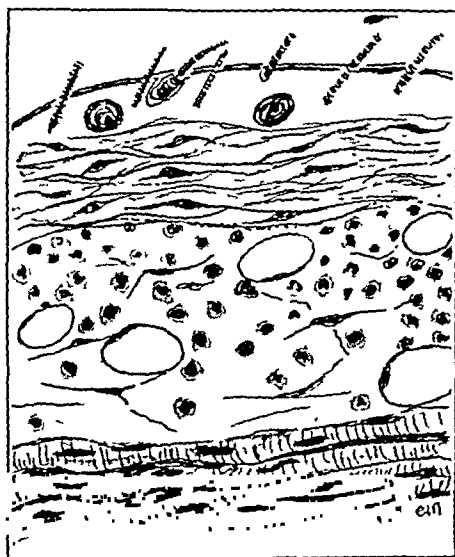


Fig. 1.

Fig. 1.—Dilute HCl, twenty-four-hour reaction. Lymphocytes and polymorphonuclear cells.

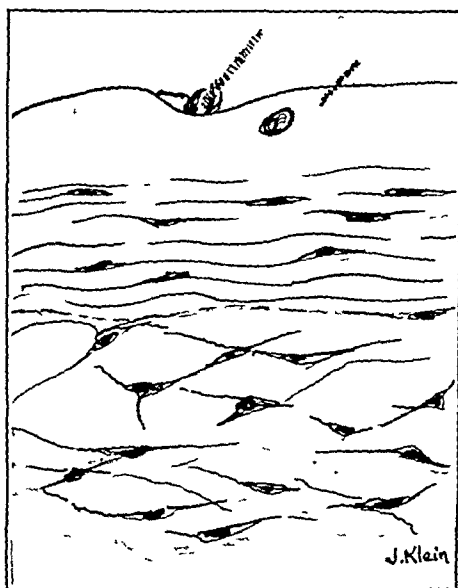


Fig. 2.

Fig. 2.—Normal skin and subcutaneous tissue after injection of 1-1,000 NaOH.

for one week; No. 5, 1 c.c. of 1-1,000 lactic acid daily for one week; No. 6 was given 1/2 c.c. of 2 per cent hog thyroglobulin daily for one week; No. 7 received 1 c.c. of diphtheria toxin (1/5 M.L.D.) daily for one week.

DESCRIPTION OF LOCAL REACTION

Microscopic study of the tissues (see drawings) revealed the following changes:

1. Dilute HCl, twenty-four-hour reaction (Fig. 1). A considerable infiltration with lymphocytes was present and a moderate polymorphonuclear reaction. A count of 100 cells in a Wright stained specimen showed 82 per cent lymphocytes and 18 per cent polymorphonuclear cells.

- 2 11,000 NaOH for twenty four hours showed no local reaction whatever (Fig 2) The normal connective tissue reticulum of the subcutaneous tissues was undisturbed
- 3 11,000 HCl daily injections for one week There was a dense leucocytic infiltration in the subcutaneous tissues with a predominance of lymphocytes and reticulum cells It was a "sterile abscess"
- 4 11,000 NaOH daily injections for one week Normal skin and subcutaneous tissue No inflammatory reaction whatsoever
- 5 11,000 lactic acid daily injections for one week There was marked lymphocytic infiltration in the subcutaneous tissue Lymphocytes predominated No unusual cells were seen
- 6 One half cubic centimeter of 2 per cent hog thyroglobulin daily for one week There was a marked infiltration of the subcutaneous tissues Intense hyperemia was due to the marked dilatation of the subcutaneous capillaries There was observed extreme predominance of lymphocytes in the tissues A differential count on a Wright stain of the tissues indicated 100 per cent lymphocytes In the areas with dilated capillaries they could be seen in various stages passing through the capillary wall (Fig 3)
- 7 Received 1 cc ($1\frac{1}{2}$ M.I.D.) of diphtheria toxin daily for one week The subcutaneous tissues showed extreme infiltration with lymphocytes and polymorphonuclear cells Many leucocytes were seen with ingested erythrocytes Necrosis of subcutaneous tissue and muscle was marked Extravasation was extreme throughout the subcutaneous tissue A differential count on the c cells gave 20 per cent lymphocytes No eosinophiles or other unusual cells were noted
- 8 A normal guinea pig skin used for control showed no unusual changes

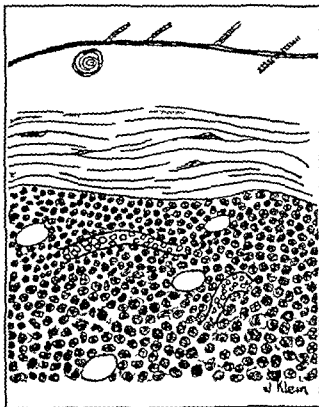


Fig 3

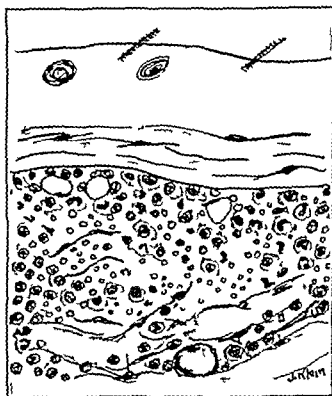


Fig 4

Fig 3—Lymphocytic infiltration after injection of thyroglobulin

Fig 4—Injection of diphtheria toxin Local necrosis hemorrhage and inflammatory reaction

THE GENERAL REACTION

The general reaction as indicated by the leucocyte and differential count corresponded very much with the local tissue reaction (see charts of blood counts, Table I)

1. 1:1,000 HCl, twenty four hours, mild leucocytosis.

2. 1:1,000 NaOH, twenty four hours, no change.

3. 1:1,000 HCl, week, moderate leucocytosis

4. 1:1,000 NaOH, week, no change

5. 1:1,000 lactic acid daily for one week. There was practically no noteworthy change in the total leucocyte count, which might be ascribed to normal variations. The differential count indicated a moderate increase in the polymorphonuclear cells.

6. One half cubic centimeter of 2 per cent hog thyroglobulin for one week. The blood count showed considerable leucocytosis with a predominance of the lymphocytes.

7. One fifth M.L.D. of diphtheria toxin daily for one week showed a marked increase in the white count with an increase in the percentage of polymorphonuclear cells.

TABLE I

GUINEA PIG NO. 1 1 1,000 HCl 24 hours' duration	PRELIMINARY COUNT						24 HOURS AFTER INJECTION					
	W.B.C.	P.	S.M.	L.M.	BAS.	TRANS.	P.	S.M.	L.M.	BAS.	TRANS.	
	6,000	5	94	0	0	1	10,100	10	89	0	1	0
NO. 2 1 1,000 NaOH 24 hours' duration	W.B.C.											
	10,400	4	91	0	0	3	10,600	45	52	0	0	3
NO. 3 1 1,000 HCl 1 week's duration	DAILY BLOOD COUNTS						1 HOUR AFTER INJECTION					
		P.	S.M.	L.M.	BAS.	TRANS.	P.	S.M.	L.M.	BAS.	TRANS.	
	5,800	28	68	3	0	1	3,000	30	67	3	0	4
	7,800	11	85	3	0	1	1,200	10	86	3	0	1
	7,000	13	82	4	0	1	2,100	8	90	2	0	0
	5,400	9	90	1	0	0	5,400	24	70	3	0	3
	9,000	23	75	0	0	2	7,000	35	65	0	0	0
	10,600	18	81	1	0	0	13,600	12	85	13	0	0
	15,600	16	83	1	0	0	14,600	12	88	0	0	0
NO. 4 1 1,000 NaOH 1 week's duration	7,800	8	92	0	0	0	5,000	14	85	1	0	0
	9,600	5	91	2	0	0	5,200	9	87	2	0	2
	11,200	26	71	1	0	0	5,000	16	83	0	0	1
	7,000	12	88	0	0	0	8,600	9	91	0	0	0
	8,600	10	90	0	0	0	9,800	25	65	0	0	0
	8,000	11	89	0	0	0	7,400	20	80	0	0	0
	9,600	20	80	0	0	0	8,400	22	78	0	0	0
NO. 5 1 1,000 lactic acid 1 week's duration	9,600	8	92	0	0	0	7,600	19	51	0	0	0
	9,400	1	96	0	0	0	10,800	16	82	2	0	0
	8,200	16	81	0	0	1	8,800	41	59	0	0	0
	9,600	60	40	0	0	0	12,800	14	85	0	0	0
	9,200	17	8	0	0	2	10,600	44	55	0	0	0
	8,600	15	84	0	0	3	8,400	16	80	1	0	1
	8,200	72	23	0	0	1	9,500	50	47	1	0	2
NO. 6 Thyroglobulin 1/2 c.c. 2 per cent daily	7,600	6	92	0	0	2	4,600	10	88	0	0	2
	6,600	18	79	0	0	2	6,000	4	94	0	0	2
	9,000	22	78	0	0	0	8,000	2	98	0	0	0
	11,800	18	82	0	0	0	10,400	11	87	1	0	1
	9,600	17	82	0	0	1	7,600	13	87	0	0	0
	9,800	20	80	0	0	0	10,800	15	83	0	0	2
	15,200	24	76	0	0	0	14,600	40	60	0	0	0
NO. 7 1 c.c. Diphth. toxin 1 week's duration	15,800	18	82	0	0	0	19,000	24	75	1	0	0
	18,000	14	86	0	0	0	15,400	54	46	0	0	0
	22,600	9	91	0	0	0	23,200	48	52	0	0	0
	24,200	33	66	0	1	0	24,800	22	77	0	1	0
	10,400	15	84	0	0	1	19,000	23	76	1	0	0
	19,400	34	66	0	0	0	18,200	40	60	0	0	0
	11,000	9	91	0	0	0	13,000	34	63	0	2	1

DISCUSSION OF RESULTS

Dilute alkali induced no change in the local or general reaction. As might be expected dilute HCl was quite irritating and provoked both a local and general reaction. Lactic acid had a greater local effect than the blood count would seem to indicate. It was interesting to find that thyroglobulin provoked a violent lymphocytic reaction and dilatation of the tissue capillaries, almost equal in extent with that induced by $\frac{1}{2}$ MLD of diphtheria toxin, although not associated with hemorrhage and necrosis, as was the latter. The most severe local and general reaction was caused by diphtheria toxin which raised the leucocyte count to 24,000 and increased the proportion of polymorphonuclear cells. Thyroglobulin induced a fair increase in leucocytes (7,600 to 15,000) and a relative lymphocytosis. The latter finding may explain the tendency to lymphoid hyperplasia in exophthalmic goiter, where even the thyroid tissues are infiltrated with lymphocytes. It seems that a substance which causes a local inflammatory reaction, usually induces a general response as shown by the blood count, although there may be exceptions as is the case with lactic acid. The most common cell reaction was lymphocytic in nature. Hydrochloric acid and diphtheria toxin affected the polymorphonuclears also. No unusual cells, such as eosinophiles or giant cells were seen. Of practical interest is the lack of reaction to alkali. When hypodermic and subcutaneous medication is given it would seem advisable to have an alkaline reaction, if possible.

SUMMARY

A comparison was made of the local and general effects of the subcutaneous injections of 11,000 NaOH 11,000 HCl lactic acid, thyroglobulin, and diphtheria toxin. The alkali produced the least and diphtheria toxin the most extensive reaction. Of particular interest was the marked lymphocytic local and general response induced by thyroglobulin.

REFERENCES

- 1 Wells H G. Chemical Pathology 1925
- 2 Heinsheimer, S. Frankfurt Ztschr f Path 39 277 1930
- 3 Gabritschewsky, G. Ann de l Inst Pasteur 4 346, 1890

3917 GLADYS AVENUE

CONSTITUTION AND ARTHRITIS*

JOSEPH KOVACS, M.D., AND EDWARD F. HARTUNG, M.D., NEW YORK, N. Y.
WITH THE TECHNICAL ASSISTANCE OF VIRGINIA HANSCOM, A.B.

THE modern American viewpoint in arthritis (as in most disease states) is topical when approaching it from the aspects of research, diagnosis, or treatment. This has definite but only partial value, as in the case of focal infection and vaccine therapy in relation to arthritis. No matter from what angle we approach the study of disease, an understanding of constitution and its relation to pathologic processes is important. However, the term constitution connotes very little that is tangible to our minds, involving as it does those imponderable quantities we call heredity, immunity, and the vagaries of the endocrine and vegetative nervous systems.

There are two factors at work in the formation of an individual's constitution. One of these, comprising the qualities inherent in the germ plasm, is called the genotypic factor. The other, the paratypic factor, expresses the effect of adverse or useful environmental forces and modifies the character of the organism from the first moment of life. These two sets of agencies are continually modeling the individual, and the final result is the patient as he presents himself to us.

In Europe, especially on the continent, great emphasis has been placed on constitution as the ultimate determining factor in disease, and its resultant pathologic picture. The viewpoint is frequently held that the same etiologic factors playing in an identical manner on varying constitutional make-ups, will produce different types of diseases, different clinically and pathologically. Here in America, while the idea is by no means foreign to us, it is too often neglected. If it is true that certain constitutions predispose to certain diseases (and this is commonly held in such conditions as tuberculosis, peptic ulcer, and gall-bladder disease), then a more complete knowledge of these various constitutions is of extreme importance in connection with such a disease as chronic arthritis.

The earlier workers on arthritis, notably Heberden (1802), Haygarth (1805), and Robert Adams (1857), neglected to observe, or at least failed to note, any association of constitutional states with the disease. The first notable mention of the subject was by Bryant and Goldthwaite¹ (1909), who pointed out the association of the light-boned, slender, asthenic, visceroptotic type with rheumatoid arthritis. As a matter of fact, such an observation only became possible after the sharp differentiation pathologically in the early part of the century between rheumatoid arthritis and osteoarthritis. Since that time, the fact has been more frequently pointed out that rheumatoid arthritis occurs predominantly in the asthenic type of constitution and osteoarthritis in the pyknic

*From the Arthritis Clinic of the Department of Medicine of the New York Post-Graduate Medical School and Hospital.

Received for publication, September 20, 1935.

type (Osgood,² Swann³) The etiology of these two diseases is still a matter of serious dispute Are they, as claimed by most, two different diseases, or, as claimed by a few, the same disease manifesting itself differently in different constitutional types?

A study of constitution in arthritis will be of value, for although it will not reveal any new facts not already described in general terms by observing physicians, nevertheless, it will present these facts on a mathematical basis There are many approaches to constitution, the most obvious ones being morphologic, physiologic, immunologic, and psychologic We have chosen the morphologic and psychologic methods of approach On the same patients on which this study is based, other factors of constitution are in the process of investigation and will be the subject of subsequent papers

The modern constitutional study is based on the acknowledgment of two types, known from ancient times as *habitus apoplecticus* and *habitus phthisicus* They represent the extreme antithetic variation from the median type We find them in every race and at every age, in women as well as in men Function, morphology, and the predisposition to disease are antagonistic in the two antithetic body types Contrary to the older intuitive method of recognizing the two constitutional types, the anthropometric method, inaugurated by Johannis Sigismundi Elsholzius,⁴ in the year 1654 and perfected by Achille di Giovanni⁵ in 1800, is based on numerical determinations Since then, Viola⁶ and Nicola Pende⁷ in Italy, Martinus⁸ Krause,⁹ and Bauer,¹⁰ in Germany, Bryant,¹¹ Bean,¹² and Draper¹³ in America and others, have perfected the technic The nomenclature in constitutional studies is confusing, as nearly every worker has his own We have used that of Boichardt,¹⁴ which is more generally accepted and which differentiates between asthenic (the old *habitus phthisicus*) and pyknic (the old *habitus apoplecticus*) type of individuals

Di Giovanni was the first who attempted to describe the morphologic configuration of those who suffered with arthritis Lack of a clear cut conception was due to the poor differentiation of the manifold rheumatic manifestations as we know them today especially the distinction between rheumatoid arthritis and osteoarthritis—the two rheumatic conditions in which constitutional differentiation is most clear cut

METHOD

We took in sequence of their arrival at the clinic, 50 cases of osteoarthritis and 50 cases of rheumatoid arthritis and subjected them to careful anthropometric and psychologic study All were females The criteria on which one bases the differentiation between rheumatoid arthritis and osteoarthritis are now fairly well understood, the main difficulty being those cases which are designated "mixed," where osteoarthritis and rheumatoid arthritis are seen at the same time in the same patient In this study we tried to include only those cases purely of one or the other type

The technic of measurement was based largely on that of Martin,¹⁵ as outlined in his *Textbook of Anthropology*, but suggestions were drawn also from the works of Draper¹³ We made a few relatively unimportant modifications Having a general idea of the different constitutional types found in chronic

arthritis, we decided on a reduction in the number of measurements, and our experience has led us to believe that we have taken all the measurements which seem to be of importance in the differentiation of constitutional types in rheumatoid arthritis and osteoarthritis. In addition, we added the span-height, upper extremity-height, lower extremity-height, chest circumference-height, abdomen circumference-height and neck length-height indices to the generally accepted neck length-neck circumference, thoracic anteroposterior diameter-thoracic lateral diameter, and cephalic length-cephalic breadth indices.

We have not compared our findings with the so-called normal type. It is very difficult in a hybrid race, such as we are dealing with in America, to establish an acceptable standard. The literature gives no help in determining normal figures. If we compare Viola's⁶ so-called normal or median measurements taken on the Venetian female, or Rott's¹⁶ median measurements taken on the German female, with Draper's¹⁷ measurements taken on the average American female, we get decidedly different results. For these reasons, we have used the method suggested by Draper, comparing various disease groups, and have tried to define the various characteristic differences.

For better comparative study, we also divided the patients into age groups of ten years, and compared the constitutional findings in these groups. Unfortunately, we had only sufficient material for comparison between the age groups of forty-one to fifty and fifty-one to sixty. The ages of the examined 50 osteo- and 50 rheumatoid arthritic individuals were as shown in Table I.

TABLE I

	AGE						TOTAL
	11-20	21-30	31-40	41-50	51-60	61-70	
Rheumatoid arthritis	2	6	16	16	10	—	50
Osteoarthritis	—	—	4	15	18	13	50

The anthropometric measurements gave the results (in centimeters) shown in Tables II and III.

Comment.—An analysis of the results of our study demonstrated that the weight of the rheumatoid arthritic is, on an average, twenty pounds less than that of the osteoarthritic individual.

Another interesting finding is that the osteoarthritic group has a longer span length than the rheumatoid arthritic group, and in many cases the span length exceeds the standing height, showing a tendency to increased horizontal measurement. We did not find marked differences between the standing height, and upper and lower extremity length in the two groups of arthritic individuals. Furthermore, we did not find pronounced difference between the head measurements, except that the osteoarthritic has a somewhat longer and slightly narrower head. On the other hand, there are marked differences in the neck length and neck circumference in the two types of arthritis. The osteoarthritic person has a large and short neck; the rheumatoid has a long and small neck. The neck of the rheumatoid is, on the average, 1.2 cm. longer and its circumference is, on an average, 2.2 cm. less than that of the osteoarthritic person. We found also a marked increase in the measurements of the thoracic anteroposterior diameter, thoracic lateral diameter, chest circumference and abdomen circumference in the

osteoarthritic group, the thoracic diameter is around 1 or 2 cm greater, the chest circumference around 9 or 10 cm larger, and the abdomen circumference around 4 cm larger. Also, the biiliac diameter is around 2 cm wider in the osteoarthritic group. We did not find pronounced differences in the umbilicus pubis and xiphoid umbilicus lengths of the two groups. The average showed some

TABLE II

ANTHROPOMETRIC MEASUREMENTS OF 50 RHEUMATOID AND 50 OSTEOARTHRITIC INDIVIDUALS

	RHEUM ARTHR.	OSTEO ARTHR.		RHEUM ARTHR.	OSTEO ARTHR.
Age	40.84	54.02	Abdomen circumference	86.26	90.62
Weight	138.87	158.24	Biiliac diameter	26.44	28.70
Span	154.34	156.38	Umbilicus pubis length	15.09	15.86
Standing height	156.61	156.47	Xiphoid umbilicus length	16.80	18.51
Upper extremity length	68.71	69.39	Cephalic length breadth index	86.65	83.02
Lower extremity length	89.19	89.70	Neck length neck circumf in dex	39.60	33.20
Cephalic length	17.84	18.16	Neck length stand height index	7.90	7.00
Cephalic breadth	15.31	15.16	Upper extrem stand height in dex	43.87	44.34
Head circumference	53.76	54.84	Lower extrem stand height in dex	56.90	57.30
Neck length	12.44	11.20	Thoracic index	75.90	75.60
Neck circumference	31.40	33.67	Spin stand height index	98.50	99.90
Thoracic ant post diameter	18.26	20.79	Abdomen circumf stand height index	55.00	57.90
Thoracic lateral diameter	25.37	26.78	Chest circumf stand height in dex	54.00	60.00
Chest circumference	54.59	94.01			

TABLE III

ANTHROPOMETRIC MEASUREMENTS OF THE AGE GROUPS OF FORTY ONE TO FIFTY AND FIFTY ONE TO SIXTY

	AGE FORTY ONE TO FIFTY		AGE FIFTY ONE TO SIXTY	
	RHEUM ARTHR.	OSTEO ARTHR.	RHEUM ARTHR.	OSTEO ARTHR.
Weight	134.53	161.62	126.19	156.82
Spin	154.80	159.50	151.77	155.22
Standing height	157.07	158.86	155.72	155.27
Upper extremity length	69.22	71.34	69.05	68.66
Lower extremity length	90.03	89.80	88.42	90.35
Cephalic length	17.87	18.19	17.86	17.89
Cephalic breadth	14.84	15.13	15.20	15.09
Head circumference	54.17	54.98	54.37	54.74
Neck length	12.21	10.65	11.78	11.14
Neck circumference	31.62	33.38	31.44	33.27
Thoracic ant post diameter	18.42	20.71	17.92	20.72
Thoracic lateral diameter	26.34	27.93	25.19	26.72
Chest circumference	94.98	96.00	86.03	92.47
Abdomen circumference	89.72	100.47	88.70	99.32
Biiliac diameter	26.94	29.17	27.84	28.66
Umbilicus pubis length	14.76	16.15	14.86	15.72
Xiphoid umbilicus length	17.18	18.48	17.24	19.21
Cephalic length breadth index	87.60	83.17	87.16	84.30
Neck length neck circumference index	38.12	31.90	37.43	33.42
Neck length stand height index	7.82	6.73	7.64	7.10
Upper extrem stand height index	44.07	44.92	44.30	44.23
Lower extrem stand height index	57.10	56.52	56.74	57.60
Thoracic index	69.92	75.58	71.14	77.56
Spin stand height index	98.52	100.07	97.43	99.96
Abdomen circumf stand height index	57.12	67.28	56.72	63.92
Chest circumf stand height index	54.17	60.17	55.23	59.50

differences, but both measurements varied so greatly in the individuals that we did not find any characteristic property which would help in the differentiation of the two groups.

Characteristic of the rheumatoid group is the comparatively greater neck length-neck circumference index. The neck length-height index does not give so marked a difference; still, this index is increased, demonstrating that neck length compared to the body length is longer in the rheumatoid group.

The upper and lower extremity-height indices, and the thoracic anterior posterior-lateral diameter indices did not show marked differences. On the other hand, the span-height index is increased and close to one in the osteoarthritic group.

The abdomen circumference-height and chest circumference-height indices are decidedly increased in the osteoarthritic group.

When we compare our anthropometric findings by age groups of forty-one to fifty and fifty-one to sixty, we get practically the same results. In the age group of forty-one to fifty the weight difference is around 27 pounds, and in the age group of fifty-one to sixty, over 30 pounds. These findings show that in the osteoarthritic group there is a tendency to fat deposit with the advance of age.

We find a decided difference between the two types of arthritis if we use the Pignet index ($X = H - (C + W)$) as a comparative measure, H representing standing height, C chest circumference, and W body weight, all in metric units.

PHYSICAL DEVELOPMENT	OSTEO-ARTHRITIS	RHEUMATOID ARTHRITIS
6 +	84%	18%
5 +	6%	12%
4 +	0	20%
3 +	8%	16%
2 +	2%	16%
1 +	0	18%

Thus we see that in osteoarthritis 90 per cent have well-developed constitutions, according to the Pignet index, compared with only 30 per cent in the rheumatoid group.

PSYCHOLOGIC ANALYSIS

An attempt was made to determine differences psychologically between the rheumatoid and osteoarthritis groups. Psychologic classification of people falling within the normal range of variation is extremely difficult, and made still more difficult by the transient contact of the investigator with ambulatory patients. We used a method of analysis devised by Dr. Smiley Blanton. The 100 cases mentioned above were classified according to this outline. A conscientious effort was made to make adequate mental contact with each patient.

An analysis of our tables shows that we were unable to demonstrate any marked difference psychologically between these two groups. Whether this is a fact or whether our analytical approach was faulty, we are unable to say. Since the tables are large and the results negative, we did not think it worth while to publish them here.

CONCLUSIONS

Anthropometric studies of 50 rheumatoid arthritic and 50 osteoarthritic patients reveal marked differences in constitutional morphology. The most outstanding are that

The rheumatoid group has a tendency to increased longitudinal measurements. Individuals of this group have decidedly longer and thinner necks, they are mostly slender, though not necessarily tall, their weight is around normal or under the average.

The osteoarthritic group has a tendency to increased horizontal measurements. Individuals of this group have shorter, thick necks, their silhouette is more massive than slender, their chest circumference in relation to abdomen circumference is comparatively greater, the thoracic anteroposterior diameter being by far the larger. They have a tendency to be overweight.

No psychologic differences were found between the rheumatoid and osteoarthritic groups.

REFERENCES

- 1 Goldthwaite, T. F. Our Present Understanding of Rheumatic Diseases, Milwaukee, 1911
- 2 Osgood, R. B. Orthopedic Aspects of Chronic Rheumatism and Arthritis, J. A. M. A 95 992, 1930
- 3 Swain, L., and Spear, L. Studies of Basal Metabolism in Chronic Arthritis, Boston M & S J 197 350 1927
- 4 Elsholzius, Johannes Sigismundi. Anthropometria, Patavia, Typis Jo. Bap. Pasquati, ninth section 1654
- 5 Di Giovanni Achille. Clinical Commentaries Reduced From Morphology of the Human Body, English Edition, London, 1919
- 6 Viola, G. La costituzione individuale, Bologna, Lucio Capelli, 1922
- 7 Pende, Nicola. Constitutional Inadequacies, Philadelphia, 1923, Lea and Febiger
- 8 Martius, F. Konstitution und Vererbung, Berlin, 1914
- 9 Krause, Fr. Allgemeine und spezielle pathologie der person, Leipzig, 1919
- 10 Bauer, Julius. Methoden der Konstitutionsforschung, Berlin, 1923
- 11 Bryant, John. The Carnivorous and Herbivorous Types in Man, Boston M & S J 172 321, 1915
- 12 Bean, R. B. Morbidity and Morphology, Bull. Johns Hopkins Hosp. 23 363, 1912
- 13 Draper, George. Human Constitution Philadelphia, 1924 W. B. Saunders Co.
- 14 Borchardt, L. Funktionelle und trophische Momente als Ursachen des gegensatzlichen Verhaltens von Pyknikern und Asthenikern, Ztschr. f. Konstitution Therap. 16 1, 1932
- 15 Martin, R. Lehrbuch der anthropologie, Fischer, Berlin, 1928
- 16 Roff, A. Körperbaustudien an deutschen Frauen, Anthropol. Anz. 3 39, 1926
- 17 Draper, George. Disease and the Man. Anglo-French Library of Med. & Biol. Sciences, London, 1930

THE EFFECTS OF DYES ON *ENDAMEBA HISTOLYTICA* IN VITRO*

H. TSUCHIYA, Sc.D., ST. LOUIS, MO.

THOUGH the effects of dyes on free-living protozoa were studied by various workers, Koch¹ was probably the first to systematically investigate the problem on parasitic protozoa. She undertook to determine dilutions of various dyes lethal to trophozoites of *Endameba gingivalis* and concluded that the action of dyes on the organisms in vitro was amebicidal rather than amebastatic. According to her investigations the phenol-methanes were found to be the least amebicidal, while the diphenyl-methanes were highly lethal to the amebas. Howitt² previously observed that acriflavine was apparently as toxic for *E. gingivalis* in vitro as certain arsenical compounds, but the inhibitory effect on the growth was lost when the dye was diluted beyond 1:142,000.

Tripoli³ introduced cysts of *E. histolytica* into culture medium containing 0.1 c.c. of 1 per cent aqueous solution of acriflavine and claimed to have succeeded in growing amebas by reducing bacterial population in the culture. Browne⁴ recently demonstrated that 1 per cent aqueous solutions of neutral red and janus green were toxic to *E. histolytica*, while trypan blue and Nile blue were not in concentrations lower than 0.5 per cent.

Since bacteria-free cultures of amebas are not available at present, the effects of dyes in reality involve both the accompanying bacteria as well as amebas present in culture. Variations in the composition and reaction of the medium as well as the accompanying bacterial flora may, therefore, be taken as factors responsible for differences in results of various investigators. Koch claimed that trophozoites of *E. gingivalis* were able to live in cultures with a minimum bacterial content, but the nature of relationship between the bacteria and protozoa was not discussed by her. That such relationship might be of importance was well brought out by Cleveland and Saunders⁵ in their study on *E. histolytica*. These authors believe that the amebas were able to multiply very rapidly in vitro when *Escherichia communior*, *Vibrio comma*, and *Neisseria catarrhalis* were present.

The present study was primarily undertaken to determine the viability of cysts and trophozoites of *E. histolytica* in vitro when exposed to selective action of various dyes; second, it was hoped that it may be possible to ascertain whether the presence of dyes may cause biologic variations of amebas, particularly the development of "dye-resistance."

MATERIALS AND METHODS

Washed Cysts.—The strain of cysts of *E. histolytica* studied came from a carrier on whom observations on "encystment cycle" were previously made.⁶

*From the Department of Bacteriology, Immunology and Public Health, Washington University School of Medicine.

Received for publication, October 3, 1935.

Washed cysts were prepared according to the method described by me⁷ and were found to contain considerably fewer bacteria than were present in the original stool

Viability Test—The use of 0.1 per cent aqueous solution of eosin to determine the viability of the cysts was found to be quite inadequate in this study. In one instance 96 per cent of washed cysts stored at 5° C. as long as 115 days were found to be unstainable, though no growth in culture mediums could be secured from this material upon repeated attempts. This seemed to indicate that, while all of the stained cysts were dead, those unstained were not necessarily viable. Throughout this study, therefore, the viability of the cysts was ascertained by subculturing on the S. C. medium⁷ with Dorsett's egg slant. The amebas in this medium usually attained an optimum growth after an incubation period of eighteen to twenty-four hours at 37° C.

Dyes and Technique—The following dyes were used in this study: (1) Triphenyl methane dyes: gentian violet, malachite green, brilliant green, pyoktanin, acid and basic fuchsin. (2) Acridine dye: acriflavine. (3) Quinoneimine dyes: neutral red, methylene blue, safranin, and brilliant cresol blue and toluidine blue. (4) Azo dye: Bismarck brown. (5) Xanthene dyes: eosin B and mercurochrome (a combination of fluorescein and mercury).

To 5 cc. of each dye dilution was added 0.1 cc. of washed cysts, and the mixture was kept at varying temperatures (5° C., 22° C., and 37° C., respectively). At varying time intervals following exposure to the dyes, 0.1 cc. of each mixture was transferred aseptically, after thorough shaking, into a tube containing 0.9 cc. of sterile nutrient broth to dilute the dye and thus prevent its further action on the amebas. One-tenth cubic centimeter of the resulting broth dilution was introduced into the culture medium and incubated at 37° C. From time to time a few drops of the sediment were withdrawn from the bottom of the culture medium by means of a pipette (graduated to tip). A single drop, which approximated 0.2 cc. of the material, was then examined immediately under the microscope with respect to the extent of growth and motility of the organisms. The degree of dye permeability in the amebas was noted in each case with the view to corroborating the permeability of the dye with the viability of the cysts in culture.

Permanent preparations of the amebas were made either by Heidenhain's iron-haematoxylin method or by the method recently described by me.⁸ Morphologic variations of the organisms, if any, were carefully looked for, and recorded.

The Gram-stained smears of culture materials were then studied with a view to ascertain the relative predominance of gram-positive or gram-negative bacteria. The relative proportion of unchanged starch granules in culture medium after incubation was considered as indicative of the extent of growth of the starch-splitting types of organisms.

To study the effect of dyes upon the trophozoites of *E. histolytica*, 0.5 cc. of each dilution of dye was added to 4.5 cc. (liquid portion) of a culture medium which maintained luxuriant growth of the amebas, thus making the final dilution one-tenth of the original. Observations were then carried out at 24, 48, and 72 hours' incubation at 37° C. Furthermore, the amebas that survived in primary culture were transferred to fresh culture medium, and then

exposed to a similar concentration of the dye as those used previously. This process was repeated from day to day in order to determine if the so-called dye-resistant strain would subsequently develop during the course of successive transfers of the organisms.

OBSERVATIONS

The results of the exposure of cysts to different concentrations of the dyes showed that after exposure to certain concentrations of the dyes, the cysts which remained viable were determined by subculturing on the culture medium. However, the growth of the organisms in the media appeared after more or less prolonged period of lag as recorded in Table I. It was observed that in these mixtures in which the lag period was the longest (amebastasis), dyes permeated into a certain proportion of the cysts. In the mixture in which the cysts were exposed to high concentration of the dyes, the viability of the cysts was lost as determined by the absence of the growth of the organisms in cultures, and the dyes permeated all of the cysts.

Amebastatic effect was best shown by gentian violet, acriflavine and malachite green (lag periods of 72 and 68 hours), and basic and acid fuchsins to a lesser extent. On the other hand, amebas grew abundantly in culture medium after the exposure to 1:100 dilution of safranin. As a matter of fact, the growth even surpassed that seen in the control tube, while in the case of neutral red, there was neither inhibition nor stimulation of the growth of the organism. The remainder of the dyes exerted amebastatic effect to an intermediate degree. Thus, following the exposure to 1:400 dilution of eosin B, 1:800 dilution of Bismarck brown, 1:1,600 dilution of brilliant green and 1:6,400 of mercurochrome, the growth of the amebas was initiated after lag period of forty-eight hours. In the case of toluidine blue, brilliant cresol blue and pyoktanin, the incubation period was found to be thirty-six hours.

Gentian violet exhibited the amebastatic effect on the cysts of *E. histolytica*. Thus, by the introduction of 1:200 dilution of the dye, the initial growth of the organisms was not observed until an incubation period of seventy-two hours was reached. The amebas in the culture medium apparently were able to maintain a good growth for at least six days without further transfer. The individual organism showed a considerable increase in size both of the cytoplasm and to a slighter extent of the nucleus. Differences in size were so significant that the identity of the organisms might be questioned by casual observers. This might be explained on the ground that the selective action of the dye was such that incidental bacteria were affected without injury to the amebic protoplasm. This action, further, resulted in the prolongation of the life of the culture, so that without transferring or changing the medium in any way, the organisms remained actively motile and undergoing division for several days (see Table I). During this period of active growth of the amebas, surviving bacteria increased in number, and the amebas gradually returned to their normal size. Transfer of this culture to fresh medium resulted in a normal growth of the organisms.

In the cultures exposed to malachite green gram-positive bacteria were inhibited, whereas in those exposed to acriflavine the growth of gram-negative bacteria was inhibited. It was noted in general that starch granules remained undigested in this medium which indicated that the starch-splitting types of

TABLE I
EFFECTS OF DYES ON CISTS OF ENDAMEBA HISTOLYTICA IN VITRO

DYES			AMFIBICIDAL ZONE (IN DILUTIONS)		AMPHASTATIC ZONE (IN DILUTIONS)		INCUBATION PERIOD (IN HOURS)	BACTERIA		AMFIBA		
GROUP	KIND							GRAM		CYTOPLASM M (MICRONS) *	NUCLEUS M (MICRONS) *	DIVISION
								NEGATIVE	POSITIVE			
Triphenyl methane	Gentian violet		1 200 >	< 1 200	< 1 200	72	+++	+	28.4	6.9	++	
	Malachite green		1 400 >	< 1 400	< 1 400	68	+++	+	25.2	6.8	++	
	Brilliant green		1 1,600 >	< 1 1,600	< 1 1,600	48	+++	+++	22.8	6.8	+	
	Pyoktamin		1 2,000 >	< 1 2,000	< 1 2,000	36	+++	+	20.7	6.2	-	
	Acid fuchsin		1 400 >	< 1 400	< 1 400	58	+++	+++	21.1	6.8	+	
Acridine	Basic fuchsin		1 1,600 >	< 1 1,600	< 1 1,600	62	+++	+	21.6	6.4	-	
	Acridine		1 200 >	< 1 200	< 1 200	72	+	+++	24.9	6.9	++	
Quinonemine	Neutral red		-	-	-	24	+	+++	21.6	6.6	+	
	Methylene blue		1 800 >	< 1 800	< 1 800	36	+	+++	20.1	6.1	-	
	Safranim		-	-	-	24	+	+++	23.3	6.0	+	
	Brilliant cresol blue		1 200 >	< 1 200	< 1 200	36	+	+++	20.7	6.1	-	
Azo	Toluidine blue		1 200 >	< 1 200	< 1 200	36	+	+++	21.1	6.3	-	
	Bismarck brown		1 800 >	< 1 800	< 1 800	48	+++	+	23.6	6.7	+	
Xanthene	Eosin B		1 400 >	< 1 400	< 1 400	48	+++	+	21.7	6.6	-	
	Mercurochrome		1 6,400 >	< 1 6,400	< 1 6,400	48	-	+	24.6	6.7	-	
	Control		-	-	-	18 24	++	++	20.3	6.4	+	

*The size of ameba was determined by dividing the sum total of length and width of the trophozoite by 2

M (Mean) was computed by using the following formula $M = \frac{\sum X \times f}{n}$ in which n indicates the number of samples (100) × the measurements of all samples and f the frequencies in which they appeared

microorganisms were probably affected by these dyes. The size of amebas was likewise increased, though much less than in the case of the organisms exposed to gentian violet.

According to Churchman⁹ dye mixtures, though inferior to any of the constituent dyes alone in bactericidal action, seem to enhance the bacteriostatic power to such an extent as to suspend the growth of the organisms over a long period. When washed cysts were first exposed to the action of a mixture of an equal volume of gentian violet and acriflavine (1:100 dilution each) at 37° C. for one hour and then inoculated into culture medium, the amebas were usually found to maintain growth as long as eight days without further transfer. It is suggested, therefore, that this procedure may serve as a means of maintaining cultures for several days. Koch claimed that though acriflavine is known to be a powerful amebicide as tested against *E. gingivalis* in vitro, it is not so effective an amebastatic agent as gentian violet.

The duration of exposure of the cysts to the action of gentian violet, acriflavine, and malachite green clearly indicated that, while the cysts were often cultivable after an exposure of as long as twenty-four hours, an optimum degree of amebastasis was obtainable by an exposure of one hour to these dyes. As was to be expected, the optimum amebastatic effect was obtainable at 37° C., and it was less at an exposure to 5° C. or 22° C.

Acid and basic fuchsins exhibited the amebastatic effect on the cysts of *E. histolytica* in the dilutions of 1:400 and 1:1,600, respectively. The growth of amebas was first observable in cultures at fifty-eight hours in the former and sixty-two hours in the latter at 37° C. The increase in the size of the organisms was, however, negligible. Furthermore, none of the cultures thus treated was found to maintain growth any longer than three days. Apparently, the bacteriostatic effect of these dyes was less than that secured by gentian violet, acriflavine, or malachite green.

Churchman¹⁰ stated that, as a rule, acid dyes were known to be much weaker in bacteriostatic action than basic dyes. This fact may explain why a higher concentration was necessary to induce amebastasis in the case of acid fuchsin than in the case of basic fuchsin.

As will be seen from results recorded on Table II, the dyes exhibited a higher degree of toxicity upon trophozoites than upon the cysts of *E. histolytica*. This is undoubtedly due to the greater permeability of the walls of the trophozoites. With respect to the intensity of toxic action, the dyes were found to be as follows: mereurochrome exhibited the greatest toxicity to the trophozoites, while acriflavine and methylene blue showed less. Neutral red, safranin, and eosin B, on the other hand, were only slightly lethal to the trophozoites.

The manner in which the trophozoites responded to the exposure to dyes in vitro is of interest. At the outset, the defensive mechanism of the organism was at work as shown by a continual attempt on the part of the organism to expel the dye particles from its cytoplasm. This process usually required from a few minutes to one hour, at which time no dyes were detectable microscopically within the amebas. In the cultures, the process of gradual regeneration of the organisms became evident as time elapsed, and eventually normal growth was resumed by the organisms. The viability of trophozoites as affected by

the dyes depends, therefore, upon the rapidity with which the dyes were eliminated from the protoplasm of ameba. In the case of gentian violet, acid fuchsin, malachite green, brilliant green, and basic fuchsin, the organisms continued to grow in the presence of these dyes as long as forty eight hours upon continued incubation at 37° C, while no growth was discernible when other dyes were used. This seemed to indicate that the action of these dyes was amebicidal rather than amebistatic. There were no appreciable degrees of amebastasis and this was a decided contrast to the condition observed in the case of the encysted stage of the organism. As a matter of fact, the life of amebas in the majority of instances was not prolonged beyond twenty four hours and the organisms soon disintegrated either owing to the overgrowth of

TABLE II

EFFECT OF DYES ON TROPHOZOITES OF ENDAMEBA HISTOLYTICA IN VITRO

DYES		LETHAL DILUTION	SUB LETHAL DILUTION USED	INCUBATION PERIOD (AT 37° C.)					
GROUP	KIND			24 HOURS		48 HOURS		72 HOURS	
				GROWTH	DIVISION	GROWTH	DIVISION	GROWTH	DIVISION
Triphenyl methane	Gentian violet	1 10,000	1 20,000	+++	+	+++	+	-	-
	Malachite green	1 10 000	1 20,000	+++	+	++	Trace	-	-
	Brilliant green	1 8,000	1 16,000	++++	+	++	Trace	-	-
	Pyoktamin	1 8,000	1 16,000	++	-	-	-	-	-
	Acid fuchsin	1 400	1 800	+++	+	++	Trace	-	-
	Basic fuchsin	1 2,000	1 4,000	+++	+	+	-	-	-
Acridine	Acridflavine	1 40,000	1 80 000	+++	+	-	-	-	-
Quinone imine	Neutral red	1 400	1 800	+++	+	-	-	-	-
	Methylene blue	1 20,000	1 40,000	+++	+	-	-	-	-
	Saffranin	1 1,000	1 2,000	++++	++	-	-	-	-
	Brilliant cresol blue	1 8,000	1 16,000	+++	+	-	-	-	-
	Toluidine blue	1 10,000	1 20,000	++	-	-	-	-	-
Azo	Bismarck brown	1 4 000	1 8,000	++++	+	-	-	-	-
Xanthene	Eosin B	1 500	1 1 600	++	-	-	-	-	-
	Mercuriochrome	1 100 000	1 200,000	+++	+	-	-	-	-

the accompanying bacteria or to an excessive accumulation of their own metabolic products. In a few instances, the bacteriostatic effect of the dyes was made quite evident through the survival of the amebas for an additional twenty four hours.

Observations on the development of increased resistance of dyes upon repeated exposures were carried out with the use of gentian violet, acid fuchsin, and malachite green. It was found that repeated exposures of the trophozoites to the action of these dyes in sublethal dilutions eventually created an apparent degree of relative resistance, which increased in proportion to the number of exposures. Thus in eleven exposures of the trophozoites to 1 20,000 dilution of gentian violet, no dye particles were found to permeate the protoplasm of the

amebas, while sixteen exposures were necessary to establish the resistance in the case of 1:800 dilution of acid fuchsin and eighteen exposures were required in the case of 1:20,000 dilution of malachite green.

DISCUSSION

The effect of dyes on the growth of amebas in culture medium seems to depend on two possible factors as suggested by Simon¹¹ in his work on dyes and bacterial development: (1) the presence of suitable receptors in the organism, and (2) the ease with which the receptors taking up the dye recover their capacities to unite with the dye. Susceptibility and resistance of the organism apparently hinge on the degree of avidity of these receptors. Dye susceptibility may change by adaptation. Thus, it is possible to change a dye-susceptible strain to a dye-resistant one by repeatedly exposing the amebas to the action of dyes such as gentian violet, acid fuchsin, and malachite green. The amebicidal action of dye in question may, therefore, be interpreted as indicating the existence of corresponding receptors in organisms with which the dye may unite.

According to Stearn and Stearn,¹² the maximum bacteriostasis is produced by the formation of the dye-protein compound. A protein is readily liberated from the combination, so that unless some other mechanism is operative in actually destroying the bacterial protein while the growth is inhibited by the dye, the organisms remain latent awaiting for the opportunity to resume the development. The same appears to hold true with the proteins of amebas as was illustrated by the use of gentian violet and acriflavine or a combination of the two. Koch stated that dyes belonging to the acridine group are most active against bacteria in protein solution. They would, therefore, be of particular value in a medium containing egg albumen.

Variations in size of amebas observed after the exposure of the organisms to the action of the dyes may be partly explained as the result of the diminution in the bacterial population in culture. So long as this influence persisted the size of amebas remained irregular, although the organisms were able to grow without further transfer. Once, however, the effect of the dyes had passed, the amebas resumed the normal dimensions and were soon crowded out by the overgrowth of bacteria in the culture medium. It may also be possible that certain strains of amebas are capable of proliferating *in vitro* irrespective of the accompanying bacteria. The question of an increased oxygen tension of the culture medium may likewise play a rôle in size variations of the organism.

Attempts have been made in the past to separate amebas in culture medium from the accompanying bacteria. The utilization of proper concentrations of chemicals to induce the growth of amebas and simultaneously to kill the accompanying bacteria has been tried out. The cultivation of the organisms under the conditions of strict anaerobiasis and the isolation of the organisms from bacteria-free liver abscesses have resulted in apparent failure. Though dyes alone may not be used as a means of securing a pure culture, at least they would be of help in inhibiting bacteria where other means of isolation are to be used. It is suggested, therefore, that by the use of a combination of these measures, one may arrive at a method by which the amebas thrive in pure culture.

SUMMARY

1 The action of high concentration of dyes on the cysts of *E. histolytica* was found to be amebicidal, but when diluted they exhibited amebastatic effect which varied in intensity with the kinds and concentrations of dyes used. Amebastasis was best shown by the use of gentian violet, acriflavine, and malachite green, and to a lesser extent by basic and acid fuchsins. Safianin and neutral red apparently failed to produce the amebastatic effect, while the remainder of the dyes exerted a more or less intermediate degree of amebastasis.

2 In the cultures where amebastasis was induced through the addition of suitable concentrations of gentian violet, acriflavine, and malachite green, there was a marked increase in the size of the organisms. This may be accounted for by the antagonistic effect of the dyes upon the accompanying bacteria. So long as the effect remained operative, the amebas in cultures were able to thrive without transfer.

3 An optimum amebastasis was obtained by exposing washed cysts to the action of the dyes or of a mixture of gentian violet and acriflavine for one hour at 37° C, prior to inoculation into culture medium. In these circumstances, the lag period was prolonged, but the development thereafter was remarkably luxuriant. It is suggested that this procedure may serve as a means of maintaining cultures for several days. The action of dyes on the trophozoites of *E. histolytica*, on the other hand, was as a rule, amebicidal rather than amebastatic.

4 By repeated exposures of the culture of amebas to dyes such as gentian violet, acid fuchsin, and malachite green in proper concentrations, it was possible to increase the resistance of the amebas to these dyes. Significant increase of the resistance was secured by eleven successive daily exposures to 1:20,000 dilution of gentian violet, upon sixteen exposures to 1:800 dilution of acid fuchsin, and upon eighteen exposures to 1:20,000 dilution of malachite green.

REFERENCES

- 1 Koch, D. A. An Experimental Study of the Effects of Dyes, Dye Mixtures and of Disinfectants Upon *Endameba gingivalis* in vitro, Univ. Calif. Pub. Zool. 29: 241, 1926.
- 2 Howitt, B. F. The Effect of Certain Drugs and Dyes Upon the Growth of *Endameba gingivalis* (Gros) in vitro, Univ. Calif. Pub. Zool. 28: 173, 1926.
- 3 Tripoli, C. J. Preparation of Culture Media for Routine Culture of Feces for Pathogenic Amebas. Proc. Soc. Exper. Biol. & Med. 26: 245, 1928.
- 4 Browne, D. C. Effect of Vital Stains in Cultures of *Endameba histolytica*, Proc. Soc. Exper. Biol. & Med. 28: 255, 1930.
- 5 Cleveland, L. R., and Saunders, E. The Virulence of a Pure Line and Source Strain of *Endameba histolytica* From the Liver of Cats and the Relation of Bacterial Cultivations, and Liver Passage to Virulence, Am. J. Hyg. 12: 569, 1930.
- 6 Tsuchiya, H. Observations on "Encystment Cycle" of *Endameba histolytica* in a Carrier, Proc. Soc. Exper. Biol. & Med. 29: 930, 1932.
- 7 Tsuchiya, H. Further Studies on the Cultivation of *Endameba histolytica* and a Complement Fixation Test for Amebiasis. J. Lab. & Clin. Med. 19: 495, 1934.
- 8 Tsuchiya, H. A Practical Staining Method for Intestinal Protozoa, J. Lab. & Clin. Med. 17: 1163, 1932.
- 9 Churchman, J. W. Bacteriostasis by Mixture of Dyes. J. Exper. Med. 38: 1, 1927.
- 10 Churchman, J. W. Inhibition of Sporulation by Acid Fuchsin, Proc. Soc. Exper. Biol. & Med. 23: 94, 1925.
- 11 Simon, C. E. The Inhibitory Action of Certain Anilin Dyes Upon Bacterial Development, Am. J. M. Sc. 147: 247, 1914.
- 12 Stearn, A. E. and Stearn, E. W. The Chemical Mechanism of Bacterial Behavior III: the Problem of Bacteriostasis, J. Bact. 9: 491, 1924.

STUDIES ON THE EFFECT OF A HIGH-SULPHUR LOW-CARBOHYDRATE DIET IN CHRONIC ARTHRITIS*

J. C. FORBES, M.A., PH.D., R. C. NEALE, O. L. HITE, M.D.,
D. B. ARMISTEAD, M.D., AND S. L. RUCKER, M.D., RICHMOND, VA.

IT HAS been shown in a previous publication (Forbes and Neale¹), that indoluria is usually present in patients with chronic arthritis, pellagra, congestive heart failure, toxemia of pregnancy, diabetes mellitus, tuberculosis, and severe lobar pneumonia. Furthermore, the indoluria more or less parallels the clinical condition, disappearing with marked improvement.

Indole may arise from any focus of infection in the body. Since in so many cases of chronic arthritis no focus of infection can be demonstrated, one is almost forced to believe that (in these cases) the origin of the indole is the decomposition of tryptophane in the intestinal tract, the indole being either formed in greater amounts than normal or allowed to pass through the liver undetoxified.

Indole is normally detoxified in the body by being converted into indican (potassium indoxyl sulphate). Therefore, sulphur is necessary for indole detoxification. Evidence that there may be a sulphur deficiency in chronic arthritis has been presented by Sullivan and Hess² and Argy,³ who have found a lowered sulphur content of the finger nails in this condition. Couturat⁴ has shown that the sulphur content of the articular cartilage is low in patients with chronic arthritis. Furthermore, various sulphur preparations have been used from time to time in the treatment of arthritis, and in many cases have led to clinical improvement. With these facts in mind, it occurred to us that indoluria might be evidence of impairment of liver detoxification resulting from a sulphur deficiency in the liver.

Thus, it seemed logical to give to arthritic patients a diet having a high proportion of sulphur amino acids and a low carbohydrate content. In addition to taking the place of proteins, thereby limiting the amount of sulphur ingested, there is evidence that carbohydrates in large amounts may have a direct detrimental effect. For example, Fletcher⁵ produced in a case of arthritis an acute exacerbation of the condition by freely feeding carbohydrates. We have obtained similar evidence indicating the harmful effect of a high carbohydrate diet.

The importance of the diet in the treatment of chronic arthritis has been emphasized by a number of investigators, and the diets recommended are as a rule low in carbohydrates. Other factors are also stressed. Fletcher and Graham⁶ feel that the vitamin B content is an important factor, since it improves the tone of the intestinal tract and prevents stasis of its contents. Pemberton⁷

*From the Departments of Biochemistry and Medicine, Medical College of Virginia. Received for publication, September 16, 1935.

This study was in part conducted under a research grant from Eli Lilly and Company, to whom grateful acknowledgment is made.

emphasizes the importance of a balanced, low calorie low carbohydrate diet Cecil⁸ recommends a low carbohydrate diet in conjunction with vaccines and other forms of therapy Langstroth⁹ has obtained from statistical studies a definite correlation between the incidence of a number of diseases, including chronic arthritis, and the amount of protective foods or vitamins in the diet The incidence of these diseases was greatest in those individuals consuming the smallest amount of protective foods Basing his therapy on these findings, Langstroth fed a low carbohydrate high vitamin diet to patients with chronic arthritis with gratifying results

The diet used in the work here reported agrees to a considerable extent with those employed by the above investigators, having in common with them a low carbohydrate intake However, we made no attempt to decrease the caloric intake, on the contrary, in all apparently undernourished patients the diet was kept at as high an energy level as feasible by the administration of readily digestible fats such as cream and butter Furthermore, the content of sulphur amino acids was quite high

In the cases here reported, treatment was confined to dietary control, together with the use of cathartics when necessary to obtain good elimination The majority of patients on this diet improved markedly Coincident with improvement, there was a decrease in the indole elimination, and finally its complete disappearance from the urine Although the best results were obtained in the treatment of rheumatoid arthritis, some typical cases of hypertrophic arthritis also showed definite improvement This was true even in one case where repeated examination of the urine failed to show the presence of indole

The diet used by us is as follows

- Meats** At least one large portion of meat, such as chicken, veal, beef, fish, etc., daily in addition to from three to five eggs, and cheese in liberal amounts
- Vegetables** Five per cent vegetables such as lettuce celery, spinach, cucumbers, cauliflower, and broccoli, together with small amounts of 10 per cent vegetables such as beets and carrots The starchy foods such as corn, rice, potatoes, etc., are entirely eliminated
- Fruits** Two large oranges, tomatoes, or one grapefruit or its equivalent daily
- Sweets** All pastries pies, cake and sweets in general are eliminated
- Bread** Bread is limited to three slices of toast a day
- Milk** At least one quart of milk or buttermilk daily, and butter in liberal amounts, especially to undernourished patients
- Alcohol** Alcoholic liquors in any form are strictly avoided

Table I gives, in abbreviated form, the results of the determination of urinary indole on patients with different types of arthritis and their response to treatment

Table II shows the changes in urinary indole during treatment in a few typical cases The results indicate a fairly definite correlation between indoluria and the clinical condition For instance, in the case of Mr. N. it will be seen that on discontinuing his diet for several weeks not only did his arthritic pains return but indole reappeared in the urine Shortly after resuming the diet he became free from symptoms and his indoluria disappeared

It is not possible from our results to state whether the beneficial effects obtained are due to the increased sulphur content of the diet, or to the increased vitamin B content, or to the diminished carbohydrate intake It is

TABLE I

DIAGNOSIS	NUMBER OF PATIENTS	NUMBER WITH INDOLURIA	NUMBER WITH NEG. INDOLE OR TRACE	NUMBER TREATED	NUMBER MARKEDLY IMPROVED	NUMBER SLIGHTLY IMPROVED	NUMBER NOT IMPROVED
Rheumatoid arthritis	24	22	2	22	20	0	2
Hypertrophic arthritis	13	10	3	8	5	1	2
Mixed arthritis	2	2	0	2	1	0	1

TABLE II

PATIENT	DIAGNOSIS	DATE	INDOLE	CONDITION
Mr. El.	Hypertrophic arthritis	4/21	1.7	Suffering considerably
		6/ 2	1.3	Unchanged
		6/ 5	3.0	Unchanged
		6/ 6		Placed on "arthritis diet"
		7/10	Trace	Stronger, less pain
		7/31	Neg.	Considerably improved
		9/18	0.2	Few pains following recent exposure
Mrs. Ar.	Rheumatoid arthritis	6/ 7	0.7	In considerable pain
		6/ 8	0.5	Unchanged
		6/ 9		Placed on "arthritis diet"
		6/19		Markedly improved
		6/23	Neg.	Free from symptoms.
Mrs. Gr.	Hypertrophic arthritis	6/20	0.7	Suffering considerably
		6/21	0.9	Unchanged
		6/22	0.5	Unchanged
		6/23		Placed on "arthritis diet"
		6/28	Neg.	Improvement remarkable
		7/11	0.3	Some reappearance of pains following laxity with diet
		7/18	Trace	Much better
		7/31	Neg.	Some pains present following recent exposure to cold and damp
		8/ 7		Pain moderately returned following temporary discontinuance of diet
Mr. Ne.	Rheumatoid arthritis	6/23	1.0	Pain very severe
		6/25	0.7	Unchanged
		6/26	1.0	Unchanged
		6/27		Pain more severe, placed on "arthritis diet"
		6/28	0.8	Feeling better
		6/30	0.7	Somewhat improved
		7/ 5	0.5	Some improvement
		7/ 6	0.1	No pain
		7/ 8	1.4	Reappearance of pain, cause unknown
		7/18	0.6	Pain slight
		7/21	0.6	Pain slight
		7/27	Trace	In good condition
		7/29	Neg.	Free from pain
		8/24	Neg.	Continued good
		10/ 1		Diet discontinued
		10/15	1.2	Pains returned, rather severe
		10/16	1.1	Unchanged
		10/17	0.8	Unchanged, returned to diet
		11/ 6	0.3	Much better, but some pain present
		12/ 6	Neg.	In excellent condition, no pain

probable that all three may be contributing factors. Since sulphur is necessary for indole detoxification, and since indoluria disappears with improvement, it is reasonable to conclude that the increased sulphur content of the diet is a factor in bringing about the clinical improvement. Furthermore, we have produced typical arthritic changes in the joints of rabbits by the injection of small amounts of indole into the joint cavity. This work will be published in detail later. The importance of vitamin B through its effect on intestinal tone and function is obvious.

The results presented here do not eliminate the possibility that chronic arthritis arises from foci of infection in the body. However, there is possibly always putrefaction in the intestinal tract of the adult, with absorption of some of the products. If the liver were unable to detoxify these substances, it would seem natural to believe that they could produce the same general results as would arise from the presence in the system of these putrefactive products resulting from foci of infection. Furthermore, if the liver allows indole to pass without detoxification, it is likely that other known or unknown toxic substances may also pass through unchanged, and play important rôles in the etiology of chronic arthritis. If these assumptions are correct, then a study of the various factors influencing the detoxifying power of the liver may throw some light on the etiology of the disease and the means for its prevention. Work is being done on this at the present time.

SUMMARY

Observations have been made on the effects of a high protein low carbohydrate diet on the clinical course of patients with chronic arthritis, together with studies of the accompanying changes in urinary indole. Definite improvement resulted in the majority of cases, the best results being obtained in those patients with rheumatoid arthritis. Especially good results were obtained in young individuals in early stages of the disease. Coincident with improvement, the indoluria which almost invariably accompanied the disease in its active stages, diminished and finally disappeared. It is suggested that indole is causally related to chronic arthritis, and that diets rich in sulphur aid in the detoxification of indole with consequent clinical improvement in the condition.

The writers gratefully acknowledge the many helpful suggestions of Dr. William B. Porter and Dr. Frank L. Apperly during the prosecution of this work.

REFERENCES

- 1 Forbes, J. C., and Neale, R. C. Studies on Indoluria. *J. Lab. & Clin. Med.* 20: 1617, 1935.
- 2 Sullivan, M. A., and Hess, W. C. Cystine Studies in Arthritis, *Proc. Am. Soc. Biol. Chem.* 1932. Published in *J. Biol. Chem.* 25: 97, 1932.
- 3 Argy, W. P. A Comparison of the Cystine Content of the Finger Nails With the Sedimentation Reaction of the Blood, *J. A. M. A.* 104: 631, 1935.
- 4 Couturat, J. Soufre et rhumatisme chronique, *Presse med.* 42: 1164, 1934.
- 5 Fletcher, A. A. Dietetic Treatment of Chronic Arthritis and Its Relationship to the Sugar Tolerance Arch. Int. Med. 30: 106, 1922.
- 6 Fletcher, A. A., and Graham, D. The Large Bowel in Chronic Arthritis, *Am. J. M. Sc.* 179: 91, 1930.
- 7 Pemberton, R., and Osgood, R. B. The Medical and Orthopedic Management of Chronic Arthritis, New York, 1934, The Macmillan Company.
- 8 Cecil, R. L. The Medical Treatment of Chronic Arthritis, *J. A. M. A.* 103: 1583, 1934.
- 9 Langstroth, L. Relation of American Dietary to Degenerative Disease, *J. A. M. A.* 93: 1607, 1929.

THE BACTERICIDAL ACTION OF IRRADIATED OIL OF PINE ON HEMOLYTIC STREPTOCOCCUS*

FRANKLIN A. STEVENS, M.D., NEW YORK, N. Y.

TO DEMONSTRATE the bactericidal properties of irradiated oils, investigators have employed one of two methods. In the early reports, bacteria were seeded on agar plates which were inverted over dishes of irradiated oil, previously exposed to ultraviolet light, so that the bacteria were exposed to the vapor from the oils during incubation. In the more recent studies of Ross,¹ Thompson and Sheard,² and of Sears and Black,³ the bacteria were mixed with irradiated lanolin, petrolatum or mineral oil. Frequent cultures of these mixtures were made and the results of these cultures compared with those on mixtures of the bacteria with oils which were not irradiated. In most of these investigations the oils and vapors from the oils have been found weakly germicidal. This germicidal property has been attributed by some to secondary radiation from the irradiated oil, but the recent studies of Harris, Bunker and Milas⁴ show that the bacteria are probably killed by peroxides formed during the irradiation of the oil. Their conclusions are based on the facts that the bacteria are not killed if quartz plates are inserted between the surface of the oil and the plate seeded with bacteria, that organic peroxides are found in vapor from irradiated oils, and that the bactericidal effect of the vapor is proportional to the increased peroxide content of the oil occurring during its irradiation.

No studies of the effect of irradiation with ultraviolet light on the bactericidal properties of essential oils have so far been found in the literature. Since the animal, vegetable and mineral oils which have been investigated have not been bactericidal previous to irradiation, and have acquired germicidal properties subsequent only to exposure to ultraviolet light, the study of this acquired bactericidal effect is simpler than a similar investigation of essential oils. Many of these essential oils are normally germicidal and the vapor from them kills bacteria seeded on agar plates inverted over dishes of oil in the incubator. Neither of the methods successfully employed in demonstrating the bactericidal properties of the irradiated oil previously studied and reported in the literature were sufficiently accurate and quantitative to satisfactorily show that an irradiated essential oil was more bactericidal than the normal oil. In searching for a quantitative method of studying the bactericidal qualities of the normal oils, it was found that if a given quantity of oil was agitated with salt solution and after eighteen hours if the salt solution was withdrawn from beneath the layer of oil, this salt solution was not only bactericidal but also in repeated experiments would kill the bacteria in a fixed quantity of broth culture within a certain limit of time. The bactericidal qualities of this salt solution were so

*From the Department of Medicine, College of Physicians and Surgeons, Columbia University, and the Presbyterian Hospital.

Received for publication, September 26, 1935.

constant with the same oil in different experiments that this method was employed for comparing the germicidal qualities of normal and irradiated oils. Although the effect of irradiation on several oils was studied, the experiments given in detail here are those done with oil of pine. It has been found that irradiated oil of pine is more bactericidal than the normal oil.

EXPERIMENTAL

The Bactericidal Action of Salt Solution Vigorously Agitated With Essential Oils—As noted in the preceding paragraphs, one of the difficulties in determining the bactericidal effects of irradiated essential oils was the accurate estimation of the difference between the bactericidal property of the normal oils and of oils which had been irradiated with ultraviolet light. The observation that salt solution shaken with an essential oil or with the active principle of an oil retained sufficient of the oil in solution to be bactericidal, solved this difficulty, inasmuch as it permitted an accurate determination of the bactericidal property of each sample of normal oil previous to irradiation. Salt solutions which had been shaken with essential oils and separated either by standing or by centrifugation were bactericidal. The germicidal action of these solutions, however, varied greatly. Two factors entered into these variations. First lots of the same oil from different manufacturers varied greatly in their bactericidal quality against the same bacterium. Second while some oils were universally bactericidal and other oils quite inert, some were more bactericidal for one variety of bacterium than for another. After a preliminary study of these variations with different oils and with different bacteria experiments were carried out with the same lot of oil and the same bacterium. In these later experiments the only variable was the effect of irradiation on the oil.

Experi I The Bactericidal Action of Salt Solution Previously Shaken With Essential Oils—Two hundred cubic centimeters of sterile physiologic salt solution were shaken vigorously in rubber capped bottles with 2 c.c. of oils of pine, orange, eucalyptus, thyme and peppermint, and with linalool, eugenol, isoeugenol and rhodinol. These mixtures were allowed to stand two days until the oils had separated from the salt solution. Cultures of pneumococcus I, *Staphylococcus aureus*, *Streptococcus viridans*, *Streptococcus hemolyticus* Friedlanderi bacillus *Bacillus leprosepticum*, and *Micrococcus catarrhalis* were grown eighteen hours in broth containing 0.1 per cent dextrose. Nine cubic centimeters of each of these cultures were centrifuged and the sedimented bacteria were suspended in 3 c.c. of each of the salt solutions previously shaken with the different oils. Cultures were made from these suspensions on blood agar plates at frequent intervals to determine the lengths of time required to kill the bacteria. The limits of viability for these various combinations of bacteria and oils are arranged in Table I. In this table (Table I and the three succeeding Tables II, III and IV), the intervals elapsing between the time the bacteria were mixed with the salt solutions and the time at which they were killed have been indicated by the letter D followed by the elapsed time in minutes or hours. If the bacteria were living when cultures were discontinued, the letter L has been used with the interval at which the last culture was made. In a few instances S with the time interval has been used to indicate scant growth.

The Exhaustion of the Bactericidal Activity of Salt Solution Through Contact With Bacteria: The object of the preceding experiments was to show that sufficient oil remained either in solution or in suspension in salt solution agitated with it to render the salt solution bactericidal. No attempt was made to standardize the number of bacteria exposed to given amounts of salt solution, so that the results obtained give only approximate information concerning the comparative bactericidal qualities of the various oils. It was observed that heavy cultures were killed more slowly than lighter growths if exposed to the same quantity of the same salt solution. Following this observation, experiments were

TABLE I

THE BACTERICIDAL EFFECT ON DIFFERENT BACTERIA OF SALT SOLUTION PREVIOUSLY AGITATED WITH ESSENTIAL OILS

	OIL OF PINE	OIL OF ORANGE (SWEET)	OIL OF EU- CALYP- TUS	OIL OF THYME	OIL OF PEPPER- MINT	LIN- ALLOOL	EU- GENOL	ISOEU- GENOL	RHO- DINOL
<i>Staphylococcus aureus</i>	D 20 hr.	D 24 hr.	L 48 hr.	L 24 hr.	L 48 hr.	D 24 hr.	D 30 min.	D 60 min.	L 48 hr.
Hemolytic streptococcus	D 1 min.	D 26 hr.	L 30 hr.	D 3 hr.	D 90 min.	D 1 min.	D 1 min.	D 1 min.	D 10 hr.
<i>Streptococcus viridans</i>	D 60 min.		Irregular	D 4 hr.	D 10 hr.	D 2 hr.	D 1 min.	D 1 min.	D 3 hr.
Pneumococcus (I)	D 20 min.		D 10 min.	L 24 hr.	D 24 hr.	D 20 min.	D 1 min.	D 1 min.	D 3 hr.
<i>Micrococcus Catarrhalis</i>	D 1 min.	D 5 hr.	Irregular	D 2 hr.	D 5 hr.	D 1 min.	D 1 min.	D 1 min.	D 10 min.
Friedlander bacillus	D 1 min.		Irregular	D 20 min.	D 24 hr.	D 1 min.	D 1 min.	D 1 min.	D 30 min.
<i>Bacillus lepi-septicum</i>	D 1 min.	L 24 hr.	D 30 min.	D 3 min.	D 10 min.	D 1 min.	D 1 min.	D 1 min.	D 10 min.

TABLE II

THE BACTERICIDAL PROPERTY OF SALT SOLUTIONS ON SUCCESSIVE EXPOSURES TO SUSPENSIONS OF BACTERIA (HEMOLYTIC STREPTOCOCCUS)

TUBE	SALT SOLUTION SHAKEN WITH LINALLOOL	SALT SOLUTION SHAKEN WITH EUGENOL	SALT SOLUTION SHAKEN WITH ISOEUGENOL	SALT SOLUTION SHAKEN WITH OIL OF PINE
I	D 1 min.	D 1 min.	D 1 min.	D 1 min.
II	D 3 min.	D 8 min.	D 5 min.	D 4 min.
III	S 10 min.	L 20 min.	D 20 min.	D 15 min.
IV	L 20 min.	L 20 min.	S 20 min.	L 20 min.

undertaken to discover if the bactericidal effect of salt solution previously agitated with oil of pine, and with eugenol, isoeugenol or linalool, could be exhausted by repeatedly suspending hemolytic streptococcus in the same solution.

Exper. II. The Exhaustion of the Bactericidal Qualities of the Salt Solution.—Two cubic centimeters of linalool, eugenol, and of isoeugenol, and 20 c.c. of oil of pine were shaken vigorously with 100 c.c. of sterile salt solution and allowed to layer by standing. Cultures of an hemolytic streptococcus recovered from erysipelas (E 37) were grown for twenty-four hours. Nine cubic centimeter lots of this culture were centrifuged and the supernatant fluid was removed from the sediment. Three cubic centimeters of salt solution previously agitated with one of the oils were pipetted onto the sediment in one of these tubes. This

TABLE III
 COMPARISON OF THE BACTERICIDAL EFFECT OF NORMAL AND OF IRRADIATED OIL OF PINE ON HEMOLYTIC STREPTOCOCCUS

SAMPLE OF OIL	DURATION OF IRRADIATION OF OIL	1 TO 5		1 TO 10		1 TO 15		1 TO 20		1 TO 25		1 TO 30	
		11 AND	NORMAL	11 AND	NORMAL	11 AND	NORMAL	11 AND	NORMAL	11 AND	NORMAL	11 AND	NORMAL
A	1 hour	D 18 hr	L 18 hr										
B	3 hours	D 10 min	D 10 min	D 5 hr	L 3 hr	D 24 hr	L 24 hr	L 24 hr	L 24 hr				
R	3 hours			D 2 hr	D 4 hr			D 18 hr	L 18 hr				
R	3 hours	D 1 min	D 1 min	D 2 hr	D 2 hr	D 18 hr	L 18 hr	D 18 hr	L 18 hr			L 18 hr	L 18 hr
R	3 hours	D 2 hr	D 3 hr										
B	3 hours			D 8 hr	L 8 hr	D 24 hr	L 24 hr	S 24 hr	L 24 hr	L 24 hr	L 24 hr		

The Exhaustion of the Bactericidal Activity of Salt Solution Through Contact With Bacteria: The object of the preceding experiments was to show that sufficient oil remained either in solution or in suspension in salt solution agitated with it to render the salt solution bactericidal. No attempt was made to standardize the number of bacteria exposed to given amounts of salt solution, so that the results obtained give only approximate information concerning the comparative bactericidal qualities of the various oils. It was observed that heavy cultures were killed more slowly than lighter growths if exposed to the same quantity of the same salt solution. Following this observation, experiments were

TABLE I

THE BACTERICIDAL EFFECT ON DIFFERENT BACTERIA OF SALT SOLUTION PREVIOUSLY AGITATED WITH ESSENTIAL OILS

	OIL OF PINE	OIL OF ORANGE (SWEET)	OIL OF EU- CALYP- TUS	OIL OF THYME	OIL OF PEPPER- MINT	LIN- ALLOOL	EU- GENOL	ISOEU- GENOL	RHO- DINOL
<i>Staphylococcus aureus</i>	D 20 hr.	D 24 hr.	L 48 hr.	L 24 hr.	L 48 hr.	D 24 hr.	D 30 min.	D 60 min.	L 48 hr.
Hemolytic streptococcus	D 1 min.	D 26 hr.	L 30 hr.	D 3 hr.	D 90 min.	D 1 min.	D 1 min.	D 1 min.	D 10 hr.
<i>Streptococcus viridans</i>	D 60 min.		Irregular	D 4 hr.	D 10 hr.	D 2 hr.	D 1 min.	D 1 min.	D 3 hr.
Pneumococcus (I)	D 20 min.		D 10 min.	L 24 hr.	D 24 hr.	D 20 min.	D 1 min.	D 1 min.	D 3 hr.
<i>Micrococcus Catarrhalis</i>	D 1 min.	D 5 hr.	Irregular	D 2 hr.	D 5 hr.	D 1 min.	D 1 min.	D 1 min.	D 10 min.
Friedlander bacillus	D 1 min.		Irregular	D 20 min.	D 24 hr.	D 1 min.	D 1 min.	D 1 min.	D 30 min.
<i>Bacillus lepiasepticum</i>	D 1 min.	L 24 hr.	D 30 min.	D 3 min.	D 10 min.	D 1 min.	D 1 min.	D 1 min.	D 10 min.

TABLE II

THE BACTERICIDAL PROPERTY OF SALT SOLUTIONS ON SUCCESSIVE EXPOSURES TO SUSPENSIONS OF BACTERIA (HEMOLYTIC STREPTOCOCCUS)

TUBE	SALT SOLUTION SHAKEN WITH LINALLOOL	SALT SOLUTION SHAKEN WITH EUGENOL	SALT SOLUTION SHAKEN WITH ISOEUGENOL	SALT SOLUTION SHAKEN WITH OIL OF PINE
I	D 1 min.	D 1 min.	D 1 min.	D 1 min.
II	D 3 min.	D 8 min.	D 5 min.	D 4 min.
III	S 10 min.	L 20 min.	D 20 min.	D 15 min.
IV	L 20 min.	L 20 min.	S 20 min.	L 20 min.

undertaken to discover if the bactericidal effect of salt solution previously agitated with oil of pine, and with eugenol, isoeugenol or linalool, could be exhausted by repeatedly suspending hemolytic streptococcus in the same solution.

Exper. II. The Exhaustion of the Bactericidal Qualities of the Salt Solution.—Two cubic centimeters of linalool, eugenol, and of isoeugenol, and 20 c.c. of oil of pine were shaken vigorously with 100 c.c. of sterile salt solution and allowed to layer by standing. Cultures of an hemolytic streptococcus recovered from erysipelas (E 37) were grown for twenty-four hours. Nine cubic centimeter lots of this culture were centrifuged and the supernatant fluid was removed from the sediment. Three cubic centimeters of salt solution previously agitated with one of the oils were pipetted onto the sediment in one of these tubes. This

mixed with different dilutions of the salt solution which had been in contact with the oil. Cultures were made on these suspensions at frequent intervals to determine the time limits of the viability of the bacteria in each solution. The conditions under which the normal control series were done were identical with those for the irradiated oil. The irradiation of the oil was the only variable. In Table III the dilutions of salt solution have been expressed as the relative proportions between the salt solution in the final dilution and the quantity of oil used in the original dilution from which these final dilutions were made.

The Adherence to Bacteria of Peroxidic Substance Extracted in Salt Solution From Irradiated Oil of Pine The observation that vapors arising from the surface of oils, previously irradiated with ultraviolet light, inhibit the growth of bacteria has been reported frequently. So far the mechanism of this inhibiting effect has not been satisfactorily explained. The inhibition to growth was at one time attributed to secondary waves of radiant energy, but numerous experiments have been devised by various investigators proving this explanation unsatisfactory. Investigations by Harris, Bunker and Milas⁴ have shown that volatile peroxides are formed in irradiated oils, and that the "bactericidal" property of the vapor from an oil is proportional to the increase in peroxide content of the oil occurring during the period in which it is exposed to ultraviolet light. In experiments undertaken in this laboratory,⁵ it was found that both peroxides and aldehydes result from irradiation of oils but, so far, proof that the peroxides or the aldehydes account for the inhibition of bacterial growth has not been obtained. On the other hand, it has been found that the peroxides formed in oil of pine, when it is irradiated, can be extracted with salt solution and adhere to bacteria suspended in the salt solution in which these peroxides have been extracted. The adherence of these peroxides to the bacteria is demonstrated in the following experiment.

Expt. IV The Extraction of Peroxides From Salt Solution by Bacteria—The peroxides, formed in oil of pine as a result of irradiation deliver oxygen slowly. In this respect they resemble the peroxides investigated by Harris, Bunker and Milas⁴. The oil has been tested directly both previous and subsequent to irradiation by adding 1 cc of N/100 HCl, 0.1 cc of a 5 per cent solution of KI and a few drops of starch solution. When these mixtures are shaken vigorously and then allowed to stand several hours, the unirradiated oil at the most develops a faint blue color, while the reaction with the irradiated oil is intense. Salt solution which has been shaken vigorously and separated from normal oil of pine usually has given a faint or negative test. Solutions separated after shaking with irradiated oil give strongly positive reactions. In the following experiments four strains of hemolytic streptococcus were grown eighteen hours, then 50 cc of each of these cultures were centrifuged. Salt solution previously agitated with oil of pine which had been irradiated previously was pipetted onto the sediments. Three cubic centimeters of the salt solution were mixed with the sediment from 50 cc of each of the cultures. The suspensions were agitated gently at intervals and centrifuged after six hours. One cubic centimeter of the salt solution which had not been in contact with bacteria, 1 cc of the supernatant solution which had been in contact with bacterial sediment, and the sediments themselves were tested for peroxides by the addition of the

quantities of acid, KI and starch used in testing oils directly. The peroxides in the salt solution were decreased after contact with streptococcus and the sediments gave positive tests. Control tubes of untreated bacterial sediment showed no peroxides.

SUMMARY AND CONCLUSIONS

In the preceding experiments, the observation that sterile salt solution which has been shaken vigorously with oil of pine retains enough of the oil either in suspension or in solution to be bactericidal has furnished a method by which the bactericidal properties of oils which have been irradiated could be compared with those of normal oils. The methods used in the past, both that of studying the bactericidal effects of vapors arising from the surface of the oils and that of mixing the bacteria directly with the oils, have proved unsatisfactory. In these experiments, in which the bactericidal qualities of oil of pine for hemolytic streptococcus were studied, it was found that salt solution agitated with irradiated oil of pine was more bactericidal than salt solution agitated with normal oil. The bactericidal quality of the salt solution was exhausted if bacteria were repeatedly suspended in the same solution. One chemical change has been observed in oils which have been irradiated, both the peroxides and the aldehydic content of the oils are increased. The peroxides formed during irradiation can be partially extracted from the oils in salt solution, and, although it has not been shown that these peroxides are bactericidal, bacteria exposed to these solutions adsorb and remove the peroxides from solution.

REFERENCES

1. Ross, A. T.: Bactericidal Property of an Ultraviolet Irradiated Petrolatum-lanolin Mixture, *Proc. Soc. Exper. Biol. & Med.* 29: 1265, 1932.
2. Thompson, L., and Sheard, C.: The Bactericidal Action of Irradiated Oils, *Proc. Staff Meetings, Mayo Clinic* 6: 690, 1931.
3. Sears, H. J., and Black, N.: The Bactericidal Properties of Ultraviolet Irradiated Petrolatum, *J. Bact.* 27: 452, 1934.
4. Harris, R. S., Bunker, J. W. M., and Milas, N. A.: Chemical Nature of Germicidal Vapors Emanating From Irradiated Oils, *J. Ind. & Eng. Chem.* 24: 1181, 1932.
5. Stevens, F. A.: The Bactericidal and Photochemical Properties of Irradiated Petrolatum and Mineral Oil, *J. LAB. & CLIN. MED.* (In press.)

RELATION OF BACTERIAL INFECTION TO LIVER INJURY *

STUDIES IN CINCHOPHEN INTOXICATION

LIO S. RADWIN, M.D., AND MAX LEDERER, M.D., BROOKLYN, N. Y.

SINCE the publication of reports of poisoning by cinchophen, many attempts have been made to produce injury to the liver experimentally in animals by means of the drug.¹⁻⁸ The results of these investigations, however, have not been conclusive as to the rôle of the drug as a liver poison. Changes in the livers of animals which have been observed have been indefinite, not regularly reproducible, and unlike those reported in cases of human poisoning. Lehman and Hanzlik¹ emphasize that by the use of doses comparable to the therapeutic dose in human beings no investigators have been able to produce in animals injury to the liver by cinchophen akin to the reported clinical picture.

In view of the unconvincing results from the simple administration of cinchophen to animals, it seems likely that where the drug is responsible for liver damage in man there may be present some peculiar susceptibility of that individual to it. This susceptibility may be the result of previous injury to the liver, dietary irregularities, the influence of bacterial activity, etc. Working on a similar hypothesis Reichle⁹ first attempted to injure the liver by chloroform before giving cinchophen to white rats. However, he failed to find on autopsy any pathologic changes of importance. Lehman and Hanzlik¹ found that in rabbits, cinchophen did not increase the impairment of liver function previously caused by chloroform or phosphorus. In a previous work,⁴ we found that incidental pulmonary infection in rats did not aggravate the toxic effects from cinchophen in massive doses while a high carbohydrate diet served to minimize them. Infectious processes taking place in the body may be of great significance however, in that they affect the liver first, and at times quite severely.⁹⁻¹² The part played by the liver as an organ of defense against foreign bodies of all types has been well reviewed by Gunn¹³ and need not be taken up here.

An etiologic relationship between damage to liver tissue by hepatotoxins and the influence of bacterial organisms upon the liver has been demonstrated by Opie.¹⁴ The latter's experiments indicate that the activity of an hepatic poison when used in doses which alone produce little change, may be so intensified by bacterial infection as to cause destruction of almost the entire hepatic parenchyma. Even bacteria such as colon bacilli, which have relatively little pathogenicity for normal animals, become virulent when the liver is injured by a poison such as chloroform or phosphorus. He showed that the resulting liver

*From the Pediatric Research Laboratory and the Department of Pathology of the Jewish Hospital of Brooklyn.

Received for publication July 29, 1935.

Aided by a grant from Schering & Glatz Inc. of New York City.

changes could not be produced by either the hepatic poison or the bacteria alone. Most important, by the combination of chloroform and colon bacilli, he was able to reproduce in dogs a lesion closely resembling that of acute yellow atrophy in man. He also succeeded in demonstrating the same lesion in another animal, the rabbit, using a different drug, phosphorus, and a different organism, the streptococcus.

In this investigation, we have confined ourselves to a consideration of the sensitizing effect upon the cells of the liver, of living bacteria circulating in the blood stream and possibly localizing in the gallbladder or liver. More than forty years ago, Blachstein and Welch¹⁵ demonstrated the persistence of colon or typhoid bacilli in the gallbladders of rabbits months or years after their injection into the general circulation. We have therefore attempted, by means of the coincident administration of cinchophen and living bacteria, to further study the deleterious action of the drug upon the liver.

METHOD

Only adult rabbits were used. Cinchophen was administered as a 10 per cent solution of the sodium salt, with beta eucain lactate. The drug was given daily, or every other day, by divided injections into the shaved thigh. Bacterial injections were given by way of the ear vein, twenty-four hour broth cultures being used. The injection of bacteria was usually begun several days after the onset of drug administration. At the end of an experiment, the animals which survived were put to death by an intravenous injection of air.

MINIMAL LETHAL DOSE

By means of graduated doses administered to different sets of rabbits, the minimal lethal dose of the cinchophen preparation was found to be 0.7 gm. of cinchophen per kilogram of body weight.

Autopsy findings in all the animals that died as a result of this procedure were essentially the same. There was congestion and hemorrhage in all the viscera. In the liver there was retention of bile in the cells in the region of the central veins and in the reticulo-endothelial cells lining the sinusoids. In the spleen, the macrophages and fixed histocytes were filled with altered blood pigment.

STREPTOCOCCUS

A large number of reported cases of cinchophen poisoning have been in patients suffering from either chronic cholecystitis or chronic arthritis. The streptococcus is the organism most commonly found in inflammation of the biliary passages¹⁶ or in chronic arthritis.¹⁷ A number of experiments were therefore performed, in each of which, animals were given cinchophen in large doses and also different strains of streptococcus. At the same time different sets of control animals were given either large doses of cinchophen alone, or the respective bacteria alone. The experiments were mostly repetitions in whole or in part of each other, varying in dose or strain of bacteria. Tables I and II describe the details of several typical experiments and give briefly the pathologic findings on autopsy.

From the tables it can be seen that attempts to modify the action of cinchophen by means of the streptococcus were unsuccessful. The liver changes re-

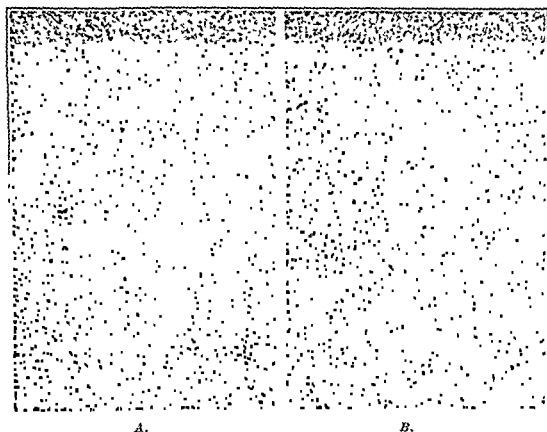


Fig. 1.—A Rabbit 39. Massive doses of cinchophen. Liver essentially normal. B Rabbit 42. Cinchophen and *Streptococcus viridans*. Architecture undisturbed. Mild granular degeneration. Increase in polymorphonuclear leucocytes.

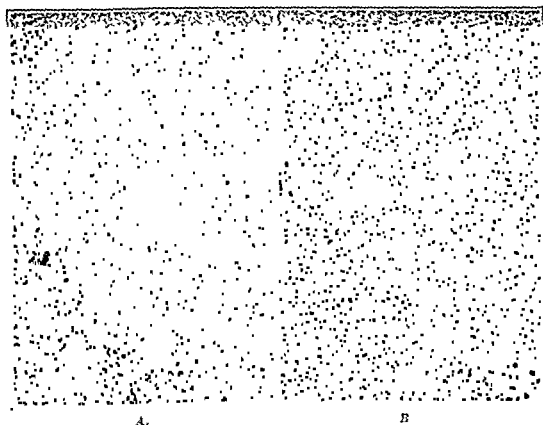


Fig. 2.—A Rabbit 47. Congestion. Miliary collection *Streptococcus hemolyticus*. Increase in number of polymorphonuclear leucocytes.

and hydropic degeneration. Rabbit 46. Cinchophen and *Streptococcus hemolyticus*. Increase in number of

sulting from the combined use of cinchophen and various strains of streptococcus were similar to, and in some cases, even less severe than those observed after the

administration of comparable doses of the same strains of bacteria alone. Similar findings were noted in the other experiments involving the use of larger or smaller doses of different strains of streptococcus with cinchophen. Cinchophen alone produced only some congestion and capillary dilatation in the liver.

TABLE I

SUMMARY OF RESULTS FROM THE COMBINED INJECTION OF CINCHOPHEN (0.3 TO 0.7 GM. PER KILO) AND STREPTOCOCCUS VIRIDANS (0.25 TO 1.5 C.C.)

	RABBIT	CINCHOPHEN INJECTIONS		NO. OF BACTERIAL INJECTIONS	RESULTS	AUTOPSY FINDINGS (LIVER)
		NO. OF INJ.	TOTAL DOSE PER KILO (GM.)			
Cinchophen	39	32	12.6	0	Died on 42nd day	Essentially Normal. Architecture undisturbed. Some capillary dilatation and congestion.
.	43	20	5.9	0	Died on 23rd day	
Cinchophen and <i>Strept. viridans</i>	41	21	6.6	5	Died on 27th day	Architecture undisturbed. Mild granular degeneration. Reticulo-endothelial cells prominent. Increased number of polys in sinusoids and capillaries. One large abscess with surrounding reaction.
	42	34	13.7	11	Killed on 46th day	
<i>Strept. viridans</i>	44	0	0	11	Killed on 36th day	Findings similar to those in animals Nos. 41 and 42, but more severe and more widespread.
	45					

TABLE II

SUMMARY OF RESULTS FROM THE COMBINED INJECTION OF CINCHOPHEN (0.3 TO 0.5 GM. PER KILO) AND STREPTOCOCCUS HEMOLYTICUS (0.25 TO 0.5 C.C.)

	RABBIT	CINCHOPHEN INJECTIONS		NO. OF BACTERIAL INJECTIONS	RESULTS	AUTOPSY FINDINGS (LIVER)
		NO. OF INJ.	TOTAL DOSE PER KILO (GM.)			
Cinchophen and <i>Strept. hemolyticus</i>	46	18	4.2	3	Died on 22nd day	Moderate capillary dilatation. Increase in polys. Slight periportal round cell and fibrous tissue reaction.
	49	21	5.5	5	Died on 26th day	
<i>Strept. hemolyticus</i>	47	0	0	5	Killed on 26th day	Marked granular and some hydropic degeneration. Congestion and capillary dilatation. Increase of polys in blood vessels and miliary collections in parenchyma.
	48					

B. COLI

Most of Opie's observations¹¹ had been based upon the administration of colon bacilli to animals receiving chloroform. A study was therefore attempted of the effect of the administration of colon bacilli with cinchophen. At the same time, to permit a comparison between the two drugs, groups of animals were given the same strain of colon bacilli and chloroform. In the chloroform studies,

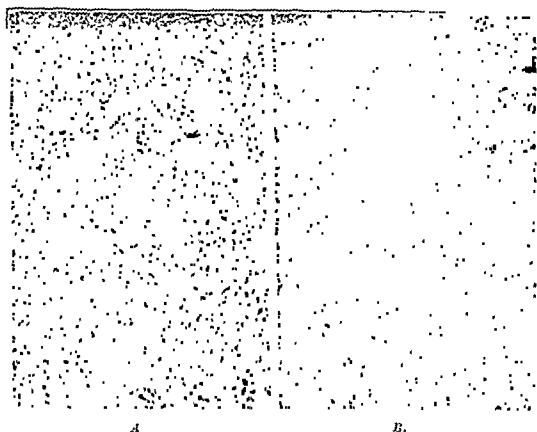


Fig 3—A Rabbit 61. Massive doses of chloroform. Architecture undisturbed. Fatty infiltration about central veins. The cells distal to this fatty layer are relatively intact. No hemorrhage. No necrosis of cells. B Rabbit 21. Chloroform and *E. coli*. Extensive destruction of cells about the central veins. Marked hemorrhage. Cellular invasion. Only a narrow fringe of relatively intact cells remains about the portal structures.

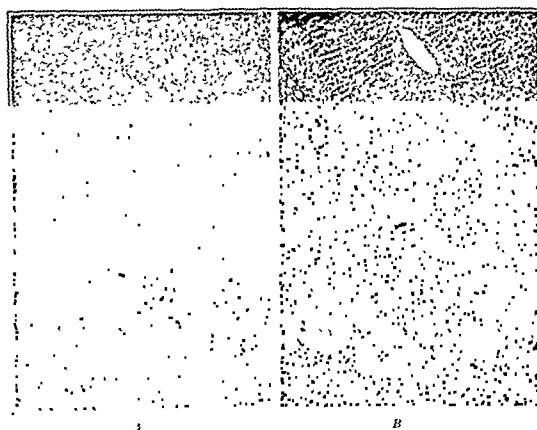


Fig 4—A Rabbit 58. *E. coli*. Granular and fatty degeneration. Congestion. Abscess formation (lower right). B Rabbit 17. Cinchophen and *E. coli*. Architecture intact. Capillary dilatation. Mild granular degeneration of cytoplasm of some of the cells. Lesion is less severe than in Animal 58.

Opie's technic was followed as closely as possible. The procedure in the main was as in the streptococcus groups. Animals were given various doses of colon bacilli, and large doses of cinchophen, or various doses of chloroform dissolved in paraffin oil. The cinchophen preparation was the same as that used before. In some experiments the bacteria were given with cinchophen or with chloroform, sixteen days after the previous administration of a single large dose of the same

TABLE III

RESULTS OF INJECTION OF CHLOROFORM (0.5 C.C. PER KILO) AND COLON BACILLI (0.5 C.C.)

	RABBIT	CHLORO- FORM	B. COLI	RESULTS	AUTOPSY FINDINGS (LIVER)
		NO. OF INJ.	NO. OF INJ.		
Chloroform and colon bacilli	16 20	4 (daily)	1	Died on 5th day	Marked destruction of cells in two-thirds of the area about the central vein, with extensive hemorrhage throughout and areas of hyaline necrosis. Adjoining tissue foamy, with evidence of hydropic degeneration. Only a narrow rim of intact cells about portal structures. Poly and fibroblastic invasion. Extensive fatty infiltration.
Chloroform	51	6	0	Died on 9th day	Fatty infiltration of about one-third of area about the central vein. Remaining tissue to the portal spaces normal. No necrosis. No hemorrhage.
	61	10	0	Killed on 24th day	

TABLE IV

RESULTS OF INJECTION OF CINCHOPHEN (0.3 TO 0.5 GM. PER KILO) AND COLON BACILLI (0.25 TO 0.5 C.C.)

	RABBIT	CINCHOPHEN		B. COLI	RESULTS	AUTOPSY FINDINGS (LIVER)
		NO. OF INJ.	TOTAL DOSE PER KILO (GM.)	NO. OF INJ.		
Cinchophen and colon bacilli	23	7	2.2	1	Died on 15th day	Architecture undisturbed. Slight granular degeneration. Capillary dilatation. Focal collections of polys throughout parenchyma. Infected thrombus in blood vessel.
	19	10	3.5	2	Died on 24th day	
	15	10	3.5	2	Killed on 24th day	
Colon bacilli	57	0	0	2	Killed on 24th day	Many small abscesses. Pronounced fatty and granular degeneration. Congestion.
	58					

organism. As controls, groups of animals were given either the bacteria alone, cinchophen alone, or chloroform alone. Tables III, IV, and V describe several experiments in detail, which are typical of the many performed.

The liver changes repeatedly seen by us after giving chloroform alone, consisted essentially of fatty degeneration about the central veins. By the coincident administration of colon bacilli with chloroform, there was produced a lesion involving extreme destruction of liver tissue and resembling that of acute yellow atrophy. These findings coincided with those observed by Opie after a similar procedure.

In the many experiments in which cinchophen was given with colon bacilli, there was noted no difference between the liver changes caused by the combination of drug and microorganism, and those caused by bacteria alone. These changes consisted of marked congestion, either fatty or granular degeneration of the cytoplasm, or a combination of the two, and at times the presence of small milium abscesses. The lesion in either case could be imputed only to the microorganisms.

TABLE V

RESULTS OF INJECTION OF COLON BACILLI AND CINCHOPHEN (0.3 TO 0.6 Gm. PER Kg.) OR CHLOROFORM (1.0 C.C. PER Kg.) SIXTEEN DAYS AFTER THE PREVIOUS INJECTION OF 0.5 C.C. OF COLON BACILLI

	FAB BIT	CINCHOPHEN		B. COLI		CHLORO- FORM	RESULTS	AUTOPSY FINDINGS (LIVER)
		NO. OF DOSES	TOTAL DOSE PER Kg. (Gm.)	NO. OF INJ.	DOSE (C.C.)	NO. OF INJ.		
Chloroform and colon bacilli	2	0		2	0.1 0.2	4	Died on 27th day	Tremendous liver de- struction similar to that found in other animals given the combination of chlo- roform and colon bacilli, e.g., Nos. 16 and 20 (Table III)
	24	0		2	0.1 0.2	4	Died on 27th day	
	13	0		2	0.1 0.2	4	Killed on 27th day	
Cinchophen and colon bacilli	14	11	3.5	6	0.05 0.25	0	Died on 39th day	Architecture undis- turbed. Marked granular degenera- tion. Congestion. Capillary dilata- tion.
	22	19	4.5	9	0.05 0.25	0	Killed on 62nd day	
	62	20	4.2	9	0.05 0.25	0	Killed on 63rd day	

COMMENT

We have been able to corroborate Opie's findings that liver changes can be induced by the combined action of bacteria and an hepatic poison, which do not resemble the changes produced by either one alone. A lesion has been produced by the use of colon bacilli and chloroform together, which is not caused by chloroform alone even in massive doses, and which closely simulates that of acute yellow atrophy. Opie has brought about similar changes by the combined use of phosphorus and *Streptococcus hemolyticus*. None of the above changes assume the characteristics of those produced by either the microorganism or the chemical poison alone, but constitute an entirely different lesion, much more severe than is ordinarily encountered.

On the other hand, when cinchophen was given together with living bacteria, there was no modification of the reaction customarily caused by the bacteria. The effect was as though no hepatic poison had been employed. In this respect, therefore, cinchophen does not act as a liver poison such as is either chloroform or phosphorus. A clear cut picture of liver destruction from the administration of cinchophen alone could not be demonstrated, nor has it been possible by our procedures to magnify the deleterious action of the drug upon the liver.

It has been the opinion of clinical observers that individuals suffering from biliary or hepatic infections are more prone to toxic disturbances from cincho-

phen. The introduction of colon bacilli into the circulation provides an infective agent which persists in the biliary passages for some time.¹⁴ Although large doses of cinchophen were given to animals in whom microorganisms, including colon bacilli, had been repeatedly injected in this way over a long period of time, no liver lesions attributable to cinchophen were encountered.

In view of the convincing nature of some of the clinical reports, it is highly probable that cinchophen does at times exert a toxic influence upon the human liver. Unless the drug is limited in its toxicity to human beings, it should be possible to reproduce these effects in animals. This has not yet been accomplished. We have been able to show in this and in previous investigations⁴ that cinchophen given in any dose does not produce an anatomic lesion in the livers of rats or rabbits. This fact, coupled with the rarity of authentic cases of poisoning, in the face of such an overwhelming number who have taken the drug in large doses, and over long periods of time without untoward effects, bespeaks a susceptibility to the drug which is not universal. On the other hand, relatively small doses of cinchophen have been employed in many of the reported cases of poisoning. In view of these facts, and of the apparently allergic nature of some of the reported symptoms,¹⁸ it is not surprising that both clinical and experimental investigators have been led to consider the question one of idiosyncrasy or allergy. This stand has recently been taken by Short and Bauer¹⁹ and by Quick.²⁰ The latter advances the interesting theory that liver necrosis, when it occurs, is a manifestation of the Arthus phenomenon.

As we stated earlier, it is not unlikely that the solution of this perplexing problem will lie in the demonstration, in individuals actually suffering from cinchophen poisoning, of a state of hypersensitivity of the cells of the liver, either because of an hereditary idiosyncrasy, or an acquired intolerance. Such a concept has also been proposed by Sulzberger.²¹ An acquired intolerance may be due to a variety of unknown factors. If one of these is the action of microorganisms upon liver cells, it is clear that unless their effect is a most unusual one, the organisms involved are probably not of the common types, since cases of cinchophen poisoning are rare, while microorganisms are frequently present in the body. Moreover, it seems evident that the mere introduction of microorganisms into the circulation is not sufficient to so sensitize liver cells as to render them susceptible to injury by cinchophen. Further studies are being conducted along the lines which we have here suggested.

CONCLUSIONS

1. The lethal dose of cinchophen for the rabbit is 0.7 gm. per kilogram of body weight.
2. Further evidence is presented to indicate the profound manner in which the coincident administration of bacteria influences the action of an hepatic poison.
3. Cinchophen given in large doses produces no anatomic effect upon the rabbit liver.
4. Cinchophen given in large doses, in combination with colon bacilli, or with various strains of streptococcus, produces no anatomic lesion in the rabbit liver.

other than that produced by the bacteria alone. The coincident administration of bacteria has failed to magnify the potentiality of cinchophen to induce damage to the rabbit liver.

5 The possibility of an unusual state of sensitization of the liver to the drug may be entertained in order to explain the clinical reports of cinchophen intoxication.

REFERENCES

- 1 Lehman, A. J., and Hunzlik, P. J. Cinchophen Toxicosis. Result of Experimental Subcutaneous and Intravenous Administration. *Arch Int Med* 52: 471, 1933.
- 2 Reichle, H. S. Cinchophen Intoxication in Rats, Arel. *Proc Soc Exper Biol & Med* 28: 581, 1931. *Am J Path* 7: 574, 1931.
- 3 Klempner, P. (Discussion of paper by Churchill and Van Wagoner. *Proc Soc Exper Biol & Med* 28: 581, 1931.) *Am J Path* 7: 574, 1931.
- 4 Radwin, L. S., and Lederer, M. Effect of Cinchophen on Albino Rat, *Arch Path* 15: 490, 1933.
- 5 Meyers, H. B., and Goodman, L. Cinchophen Hepatitis, Experimental Study, *Arch Int Med* 49: 946, 1932.
- 6 Barbour, H. G., and Fisk, M. E. Liver Damage in Dogs and Rats After Administration of Cinchophen, Ethyl Ester of Paramethylphenyl Cinchomonic Acid (Tolysin) and Sodium Salicylate, *J Pharmacol & Exper Therap* 48: 141, 1932.
- 7 Knoble, R. M., and Smith, H. A. The Effect of Cinchophen on the Liver of White Rats, *Am J Physiol* 97: 537, 1931.
- 8 Jaffe, R. Anatomie und Pathologie der Spontanerkrankungen der Kleinen Laboratoriumstiere. Berlin 1931, Julius Springer.
- 9 Buxton, B. H., and Torrey, J. C. Absorption From the Peritoneal Cavity. Part II. Absorption of Typhoid Bacilli, *J Med Research* 15: 18, 1906.
- 10 Arima, R. Das Schicksal der in die Blutbahn geschickten Bakterien, *Arch f Hyg* 73: 265, 1910, 1911.
- 11 Manwaring, W. H., and Coe, H. J. Endothelial Opsonins, *J Immunol* 1: 401, 1916.
- 12 Drinker, C. K., and Shaw, L. A. Quantitative Distribution of Particulate Material (Manganese Dioxide) Administered to Dog, Rabbit, Guinea Pig, Rat, Chicken, and Turtle. *J Exper Med* 33: 231, 1921.
- 13 Drinker, C. K., Shaw, L. A., and Drinker, K. R. Deposition and Subsequent Course of Particulate Material (Manganese Dioxide and Manganese Metasilicate) Administered Intravenously to Cats and Rabbits. *J Exper Med* 37: 829, 1923.
- 14 Gunn, J. A. Cellular Immunity: Congenital and Acquired Tolerance to Non-Protein Substances, *Physiological Rev* 3: 41, 1923.
- 15 Opie, E. L. On the Relation of Combined Intoxication and Bacterial Infection to Necrosis of the Liver, Acute Yellow Atrophy and Cirrhosis. *J Exper Med* 12: 367, 1910.
- 16 Blackstein, A. G., and Welch, W. H. Intravenous Inoculation of Rabbits With the Bacillus Coli Communis and the Bacillus Typhi Abdominalis, *Bull Johns Hopkins Hosp* 2: 96, 1891.
- 17 Brown, R. O. Etiology of Cholecystitis. *Arch Int Med* 23: 185, 1919.
- 18 Gray, J. W., Hendrick, L., and Gowen, C. H. Rheumatic Fever and Rheumatoid Arthritis From Laboratory Point of View. *Texas State J Med* 28: 317, 1932.
- 19 Parsons, L., and Harding, W. G. Cinchophen Administration. Jaundice as an Untoward Effect, Report of Cases, *California & West Med J* 37: 30, 1932.
- 20 Short, C. L., and Bauer, W. Cinchophen Hypersensitiveness. Report of 4 Cases and Review, *Ann Int Med* 6: 1449, 1933.
- 21 Quick, A. J. Probable Allergic Nature of Cinchophen Poisoning With Special Reference to Arthus Phenomenon and With Precautions to Be Followed in Cinchophen Administration. *Am J M Sc* 187: 115, 1934.
- 22 Sulzberger, Marion B. Practical Med Series (Dermatology and Syphilology), Chicago 1933, Year Book Publication p. 89.

LUNG ABSCESS*

A CLINICAL CONSIDERATION OF 101 CASES

HAROLD C. LUETH, PH.D., M.D., AND DON C. SUTTON, M.S., M.D., CHICAGO, ILL.

THERE is much confusion concerning the term lung abscess, so that four diagnostic standards were arbitrarily chosen for this study. They were: (a) aspiration of considerable amounts of pus followed in many instances by the localization of the cavity by the bronchoscope, (b) an x-ray film showing an irregular dense air shadow over circumscribed fluid, (c) the expectoration of large quantities of foul-smelling sputum particularly after a suggestive history, and (d) postmortem records. Some cases had only one of these features, while the majority had two or more. The present series was selected from a group of more than 160 patients discharged with a diagnosis lung abscess from the Cook County Hospital during the years 1933 to 1934. No case of tuberculous abscess was included in the group. Many small and early abscesses have been rejected for want of specific diagnostic points, and it is freely admitted this selection of cases tends to give a gloomier picture of the disease than is warranted. With the exception of three cases of multiple abscesses all were classified as solitary abscess.

ETIOLOGY

Incidence.—There were about 700 medical admissions for every case of lung abscess.

Age.—The number of lung abscesses increases by decades until the fifth, then it falls rapidly. Ages ranged from eleven days to sixty-six years. More than half the cases occurred in patients between thirty and fifty years. Mortality rates and incidence paralleled one another, so that about one-half the patients in each decade died (Fig. 1).

Sex.—The disease occurred more than three and a half times as often in men as in women (Table I).

TABLE I
INCIDENCE OF SEX AND COLOR

	SEX	IMPROVED	SAME	DIED	TOTAL
White	Male	37	7	29	73
	Female	5	2	3	10
Colored	Male	7	1	6	14
	Female	2		2	4
Total		51	10	40	
Aggregate					101

*From the Department of Medicine, Northwestern University Medical School, and the Medical Division of Cook County Hospital.

Received for publication, September 16, 1935.

Color—There were 83 white and 18 colored patients studied. Nearly 35 per cent of all medical admissions are colored, thus relatively more white than colored people have the condition.

Predisposing Causes—Upper respiratory infections preceded 30 per cent of lung abscesses (Fig 2). Two to six weeks, most commonly three, elapsed from the acute infection until signs of abscess were seen. No localizing sign

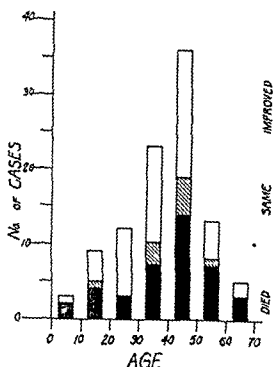


Fig 1—Age incidence

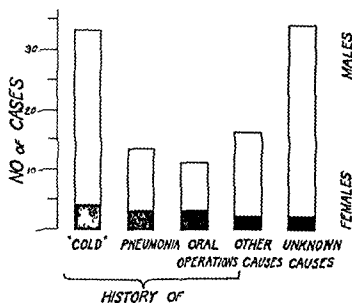


Fig 2—Predisposing causes

or symptom of these infections indicated later abscess formation. Pneumonia preceded abscess in 13 per cent of all patients. The abscess always involved the same lobe or lobes as the pneumonia, being diagnosed after an interval of three to five months. Operations about the mouth and throat were responsible for 11 cases. Almost uniformly two and a half months elapsed between the operation and the onset of profuse expectoration. Miscellaneous causes were noted in 16 cases, while no definite etiologic factor could be assigned in 31 cases.

LOCATION OF THE ABSCESS

Abscesses occurred in every lobe of the lungs (Fig. 3). Eighty-seven were unilocular whose location was fixed by the x-ray or the bronchoscope. Either the lack of diagnostic plates or failure to use the bronchoscope left 11 undetermined. Location of abscesses was given in thirds of the lung fields as seen in the x-ray films, rather than the usual terminology, lobes of the lungs. Multiple abscesses were uniformly fatal.

SYMPTOMATOLOGY

Expectoration was by all odds the most common and significant complaint. It was present in 95 cases, and quite profuse in most of them. The color varied with the type and severity of infection, the ease of bronchial drainage and the mode of treatment. Hemoptysis was the chief symptom in 19 cases; 10

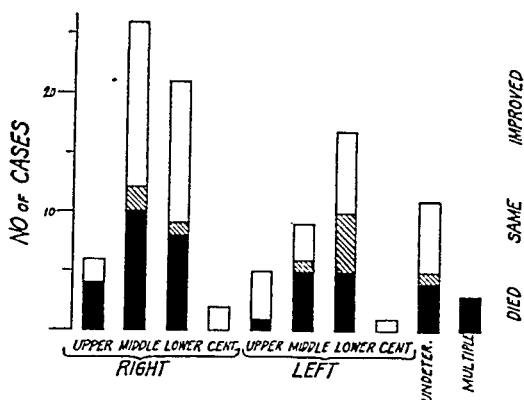


Fig. 3.—Location of abscess.

patients recovered while 9 died. Sudden massive hemorrhages were responsible for 2 deaths, and a third patient bled intermittently for seventy-two hours before he died.

The position of the abscess with respect to a bronchus frequently determined the nature of the symptoms. Early, with the formation of the abscess walls, there was usually an obstruction to a bronchus. A troublesome cough with scant thick secretion was common. Maturation of the abscess with softening of the bronchial plug followed. A sudden gush of putrid, bad-tasting material in the mouth after a slight cough indicated the beginning of drainage. In many cases this discharge of pus continued until the abscess became a cavity whose walls collapsed and healing took place by fibrosis. Intermittent expectoration of pus meant intermittent drainage. Persistently large amounts of expectoration were results of chronic abscess cavities.

Pain in the chest was a common symptom occurring in 55 cases. A moderately sharp pleural pain that radiated down the side of the chest, particularly down the midaxillary line, was a very frequent complaint. In a number

of cases confusion arose when there was pain over both chests, these patients had additional pathology on the side opposite the abscess. Neither central nor upper lobe abscesses caused pleural pain.

Loss of weight and strength was striking and was specifically mentioned in 42 cases. They were more dependent upon the duration of the disease than upon the etiology or location of the abscess.

Fever was present in almost every case. It began as a slight elevation in temperature either once or twice daily. An encapsulated abscess always showed the febrile changes of sepsis. The course of the fever was then determined by drainage. With rupture of an abscess and free drainage, the temperature fell to normal. Recrudescences meant partial obstructions and toxic absorptions. Dyspnea occurred infrequently. Other symptoms were recorded, but they were the results of complicating conditions.

PHYSICAL EXAMINATION

Three important findings of a lung abscess are dullness râles, and altered breath sounds (Table II). In 60 per cent of the entire group dullness to percussion was elicited. Râles were heard in 56 per cent, the fine moist type predominating. Nearly one third of all patients had diminished breath sounds, 17 harsh to increased, 12 amphonic and 11 bronchial. Other signs were found, but they were variable and inconstant. This is in accord with the studies of Norris and Landis¹ who found signs of cavity in 8 patients from a group of 63.

TABLE II
PHYSICAL FINDINGS

		T F FFM		PERCUSSION				BREATH SOUND				OTHER FINDINGS	
		INCREASED	DECREASED	DULLNESS	FLATNESS	HYMANY	CLACKED POT	AMPHONIC	BRONCHIAL	INCREASED	DECREASED	TELEGRAPHIC	TRICHOIN SOUND
Right	Central	1		4			1	1	1				2
	Upper	1	5	6	3			2	2			1	17
	Middle	1	4	12	6			3	2			1	16
Left	Central	1	2	4		1			2				4
	Upper	1	1	6	3			3	1			1	6
	Middle	1	7	14	2			2	1				10
Total		6	19	40	14	1	1	12	11	1		3	58

In general the earlier the lesion the fewer the physical findings. Often areas of dullness were seen to increase in size, breath sounds became more suppressed and tactile fremitus decreased as the abscess appeared. Then following rupture, amphonic breathing, increased tactile fremitus and loud moist râles were heard. All signs decreased with healing, although when a chronic cavity formed, they persisted unimpaired.

LABORATORY EXAMINATION

Urinary changes were those of fever. A moderate secondary anemia was discovered in those patients with chronic slight hemoptyses or with frank hemorrhages. The leucocyte count furnished an excellent prognostic guide. High leucocyte counts, 25,000 or more, early in the disease were generally favorable; low ones unfavorable



Fig 4

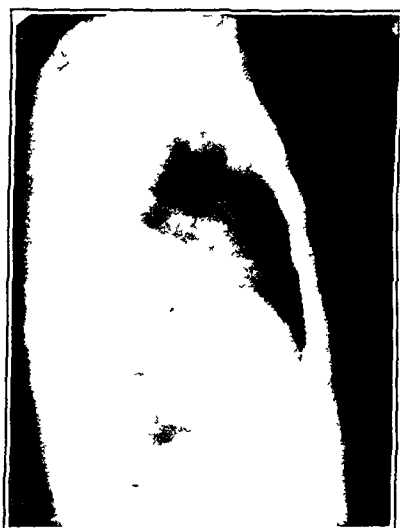


Fig 5.

Fig 4—Large abscess in the middle third of the right lung prior to rupture.

Fig. 5—Lateral view of the patient showing partial evacuation of pus with a fluid level.



Fig. 6.—Same patient showing complete healing of the abscess

ROENTGEN RAY EXAMINATION

A definite fluid accumulation within the parenchyma of the lung surrounded by a dense air shadow, both encapsulated by fibrosis, is generally

recognized as the x-ray finding of a lung abscess. While admitting that the above description is a positive method of diagnosis, yet this represents but a single phase of the disease. Other phases have received too little attention. The first indication of a lung abscess is an increased density in the lung field. Without a significant history this is often called an area of infiltration, consolidation, or fibrosis and the real process overlooked, or the pus of an abscess may have the same density as the surrounding consolidation and escape detection for many days.² As the abscess matures, the changes seen on the x-ray films have more meaning. A sharper margin limits the process from the neighboring lung substance (Fig. 4). With the onset of suppuration an increased density of the periphery is noted. Liquefaction and rupture of the abscess follow. Pus is discharged into the bronchi and expectorated. Air fills the partially evacuated cavity, giving the fluid level, characteristic of a lung abscess (Fig. 5). Complete emptying of the liquid contents of a cavity is followed by a collapse of the walls. With healing there is a progressive shrinkage of the shadow until obliteration is complete. After a variable length of time the last traces of this process disappear (Fig. 6).

BRONCHOSCOPIC EXAMINATION

One third of the patients in this study were given bronchoscopic examinations by the Ear, Nose and Throat Department, following a modification of the technique of Jackson.³ A swelling of the affected bronchial mucous membrane or extensive granulation tissue was seen. In addition a thick plug of inspissated pus occluding the bronchus was observed in many patients, but the repeated aspiration of pus from the involved main bronchus assured the diagnosis.

DIAGNOSIS

Patients who complain of pain in the chest and expectoration a few weeks after an acute upper respiratory infection, a pneumonia or an operation about the nose and mouth should be suspected of having a lung abscess. In early cases inconclusive physical signs and localized areas of infiltration as seen on roentgenograms are strongly presumptive evidence. Since the diagnosis is not always easy to make all patients with a suspicious history deserve a careful physical examination, serial x-ray films and a bronchoscopic examination.

DISCUSSION

Many causes have been responsible for lung abscesses. In an eleven day old infant a large solitary infected congenital cyst was found at postmortem. This was a rare cause. The question of aspiration of foreign bodies⁴ and infective emboli from tonsillectomies^{5, 11} has been fully discussed by others. Particularly significant was the work of Ochsner and Nesbit¹² who found that after a peritonsillar infiltration with procaine, iodized oil taken into the pharynx passed directly into the tracheobronchial tree.

Occasionally pus is aspirated from an upper lobe abscess into a lower bronchus. Heavy doses of codeine or opium so decrease the cough reflex that pus is allowed to gravitate into the lower bronchi without being expectorated. Faulkner¹³ has called this "internal drainage," and he has shown that oil easily flows from one lobe of the lung to another, or even to the opposite lung after changes in posture. In some patients this process may be so slight that an early aspiration during an exploratory bronchoscopic examination will prevent secondary atelectasis of a lower lobe.

Infection of the lungs secondary to a process elsewhere is given as a common cause of lung abscess. While it is true that upper respiratory infections are common and lung abscesses rare, relationship between the two should not be considered casually. The high mortality (Table I) and the protracted disability of those that get well¹⁴ should make us wary of this sequela of the "common cold." Pneumonias on the other hand are always considered serious, so that the patients are more carefully watched during the convalescent periods. An increase or a purulent sputum in the absence of signs of empyema, is quickly recognized as a lung abscess. Amebiasis was responsible for one case in this series, in which the hepatic abscess ruptured through the diaphragm. Secondary lung abscess after hepatic abscess perforations are often mentioned by the French.^{15, 16} This explains the frequency with which they mention emetine as a curative drug for lung abscesses.

Obstruction to a sizeable bronchus as well as infection is necessary for abscess formation. Any infection of the parenchyma of the lung with free drainage would result in a capillary bronchitis. Phagocytosis of the invading organisms and swelling of the mucous membranes would be followed by the accumulation of exoriated mucous membranes and débris in the lumen of the bronchi and bronchioles. The bechic blast and the tussive squeeze would force this material^{17, 18} into the larger bronchi where the ciliary activity and the cough reflex would expel it. Van Allen¹⁹ and others found that the absence of cough tended to give a more chronic lesion in the experimental animal. Thick pus, great swelling of the mucous membranes, or interference with the bechic blast and the tussive squeeze plug the bronchus. The trapped bacteria within the lung tissue multiply and go through the same phases as an abscess elsewhere. Inflammatory changes must extend to the pleura as pleural pain is so frequent and early a symptom. The digestive activity of the leucocytes softens the offending plug or thins it, so that it is no longer able to occlude the lumen of the bronchus. Erosion of the bronchial wall is a common mechanism, while rupture into the pleural cavity is an uncommon and serious complication.²⁰ Rarely, it may produce a bronchial fistula.

Drainage is then established. Progress from this point depends upon the character of the abscess walls. In thin-walled cavities evacuation of their contents results in the collapse of the walls. Healing takes place by fibrosis. Thick walls prevent this collapse and perpetuate this condition even though the cavity is empty.

Except for Jackson¹⁸ and a few others^{21 22} most authors have considered lung abscess as a static or stationary clinical entity. Progressive changes are easily seen in serial x-ray films, and they seem to parallel those described occurring in tuberculous cavities.²³ Surgeons, in particular, are liable to make this error, probably because they operate upon so many chronic cavities. As soon as it was recognized that pain in the right lower quadrant of the abdomen with tenderness, rigidity, nausea, and vomiting meant an acute appendicitis, fewer cases were allowed to perforate and abscess formation was infrequent. When it is realized that dullness, râles, and suppressed breath sounds two or three weeks after an upper respiratory infection or pneumonia probably mean a lung abscess an important step in the diagnosis of this condition will have been made and fewer cavities will be allowed to develop thick rigid walls.

SUMMARY

1 An analysis of 101 cases of nontuberculous lung abscess is presented, whose ages ranged from eleven days to sixty six years.

2 The following predisposing causes were given: upper respiratory infections 30 per cent, pneumonia, 13 per cent, oral operations, 11 per cent, and other causes 16 per cent.

3 Solitary abscesses were found in every part of the lung, but they occurred most often in the right middle and lower thirds.

4 Three important early physical signs are: dullness, râles, and suppressed breath sounds.

5 Successive changes seen on serial x-ray films are discussed as an early means of diagnosis.

6 Bronchoscopic examination and aspiration should be done early in every case of lung abscess.

7 Earliest signs of the condition are stressed in an endeavor to promote more effective treatment.

636 CHURCH STREET EVANSTON ILL.
30 N. MICHIGAN AVENUE CHICAGO ILL.

Without the numerous suggestions and pleasant cooperation of Dr. Thomas C. Galloway of the Ear, Nose, and Throat Department much of this work would not have been possible. I wish to thank him as well as Dr. C. H. Warfield of the Department of Roentgenology for the many favors they have given.

REFERENCES

- 1 Norris, G. W. and Iandis, H. R. M. The Diagnosis of Pulmonary Abscess, *Trans. Assn. Am. Phys.* 28: 302, 1913.
- 2 Sante, L. R. The Chest. *Ann. Roentgenol.* 11: 259, 1930.
- 3 Jackson, Chevalier. *Peroral Endoscopy and Laryngeal Surgery*, *Laryngoscope* 1915.
- 4 Adams, W. F. Experimental Bronchogenic Pyogenic Abscess. *Proc. Soc. Exper. Biol. & Med.* 31: 889, 1934.
- 5 Cherry, H. H. The Best Treatment of Nontuberculous Pulmonary Abscess, *Am. Rev. Tuberc.* 25: 634, 1932.
- 6 Tuttle, W. M. A New Method for Production of Experimental Abscesses in the Lung of Dogs. *Proc. Soc. Exper. Biol. & Med.* 30: 462, 1932.
- 7 Pierson, P. H. Comparison of Pathologic Changes in Embolic Tuberculosis and Pyogenic Abscesses. *Am. Rev. Tuberc.* 27: 150, 1933.
- 8 Frank, I. W. Pulmonary Abscess, *Ann. Surg.* 95: 675, 1932.

9. Harrington, S. W.: The Surgical Treatment of Bronchiectasis and Lung Abscess, *Am. Rev. Tuberc.* 24: 612, 1931.
10. Clerf, L. H.: The Bronchoscopic Treatment of Bronchiectasis and Lung Abscess, *Am. Rev. Tuberc.* 24: 605, 1931.
11. Holman, E.: Etiology of Postoperative Pulmonary Abscess, *Ann. Surg.* 83: 240, 1926.
12. Ochsner, A., and Nesbit, W.: Pulmonary Abscess Following Tonsillectomy, *Arch. Otolaryng.* 6: 330, 1927.
13. Faulkner, W. B.: Internal Drainage, *Am. J. Surg.* 12: 27, 1931.
14. Lueth, H. C.: The Treatment of Lung Abscess as Judged by 101 Cases, *Ill. Med. J.* 68: 440, 1935.
15. Lavergne, V. de, Abel, E., and Debenediti, R.: *Abcès Amibiasique du Poumon*, Paris méd. 1: 519, 1930.
16. Labbe, M.: *Abcès du Poumon probablement Amibiasique Guéri par L'émétine*, *Presse méd.* 38: 993, 1930.
17. Jackson, Chevalier: The Mechanism of Physical Signs With Especial Reference to Foreign Bodies in the Bronchi, *Am. J. M. Sc.* 165: 313, 1923.
18. Jackson, C., and Jackson, C. L.: Peroral Pulmonary Drainage, *Am. J. M. Sc.* 186: 849, 1933.
19. Van Allen, C. M., Fox, R. A., and Cole, H. G.: Cough as a Factor in Chronicity of Experimental Lung Abscess, *Proc. Soc. Exper. Biol. & Med.* 24: 485, 1927.
- Carlson, H. A., Ballou, H. C., Wilson, H. M., and Graham, E. A.: The Effect of Phrenicotomy Upon the Efficiency of Cough and Upon Elimination of Lipiodol From the Lungs, *Proc. Soc. Exper. Biol. & Med.* 30: 292, 1933.
20. [redacted], A. E., and Thurston, H. F.: The Treatment of Bronchial Fistulas, *J. A. M. A.* 68: 689, 1927.
21. [redacted], G. O.: Treatment and Management of Nontuberculous Pulmonary Abscess, *Arch. Otolaryng.* 19: 684, 1934.
22. Link, Elmer H.: Lung Abscess in Oxford Monographs—Diagnosis and Treatment 5: 1928.
23. Bissell, F. S.: Metamorphosis of Tuberculous Lung Changes as Observed in Serial Roentgenograms, *J. A. M. A.* 89: 936, 1927.

LABORATORY METHODS

A NEW, ONE MINUTE METHOD FOR THE STAINING OF SPIROCHETES, SPIRILLA, SPERMATOOZOA, AND RELATED ORGANISMS*

ARNOLD J. GELARIE, M.D., NEW YORK, N. Y.

IN THE experimental study of spirillosis and spirochetel diseases, it is often necessary that the blood of a large number of animals be examined at frequent intervals. Not only in such experimental studies, but also in the clinical examination of lesions of doubtful origin, is the physician called upon to employ some staining method as an aid toward identification. Of the present day methods, dark field examination is widely used. While this is highly satisfactory in the hands of an experienced technician, the general practitioner or casual laboratory worker frequently encounters difficulty. For this reason, some prefer to use Buri's India ink method. The latter is unsatisfactory, however, because of the following reasons:

- 1 Colloidal state of the ink,
- 2 Destruction of the colloidal state upon addition of salt solutions and resulting coagulation,
- 3 Presence of microorganisms and fungi,
- 4 Difficulty in making an even spread,
- 5 Breaks occurring in the smear simulating spirilla,
- 6 Difficulty in searching for single organisms

It is obvious that with Buri's method, duplication of results is impossible. Other available methods such as Levaditi's silver impregnation or Giemsa's stain are cumbersome, complicated, time consuming, and require special technical knowledge.

The method herein described is the outcome of an effort to overcome the obstacles mentioned above. Numerous and exacting tests have proved it to be highly satisfactory, all conditions are sharply defined and duplication is possible at all times.

Directions for the preparation of the necessary solutions are as follows:

- 1 Fixing solution. Dissolve 2.5 gm of recrystallized zirconyl chloride ($ZrOCl_2$) in 100 cc of 10 per cent sodium chloride solution.
- 2 Citric acid solution. Make a 10 per cent solution of recrystallized citric acid in distilled water †.
- 3 Staining solution. Dissolve 0.25 gm of gentian violet in 100 cc of N/10 sodium lactate solution. The pH of this mixture should be approximately 8.4. If not, it should be so adjusted.

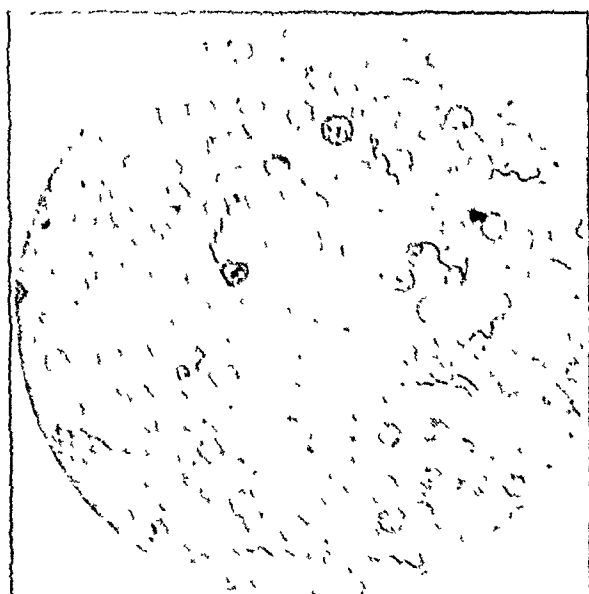
*Received for publication August 28, 1935.

†To avoid fungus formation add a few crystals of thymol.

1. *Solution of K_2HgI_4* Dissolve 0.5 gm. of mercuric iodide in 100 c.c. of 0.5 per cent potassium iodide solution.

5. *Counterstain* Dissolve 0.2 gm. of methylene blue in 100 c.c. of distilled water and add 0.2 gm. of carbolic acid.*

The material to be examined is smeared on the slide in a thin layer and allowed to dry in the air (not over the flame). Cover with the zirconyl chloride solution, allow to stand five minutes and then wash with water. The zirconyl chloride solution acts as a fixative, replacing fixation over the flame. Then place a few drops of the 10 per cent citric acid solution on the smear, allow to remain for ten seconds and wash again with water. The slide is now ready for staining. A few drops of the staining solution are placed on the slide, allowed to remain for thirty seconds and then washed off with water. This is followed by momentarily



maintained. The red cells show a finely granular rose colored cytoplasm, while the chromatin of the white cells stain blue. On the other hand, the spirilla are stained a deep purple,* differentiating them easily from surrounding tissue.

The *Treponema pallidum* obtained from the "Reizserum" differs from the above, in that formed elements are usually absent. The organism is stained a deep purple* and is very sharply defined in the clear, colorless field or in the faint, pink base formed by the stained coagulated serum.

With spermatozoa, to obtain exact detail, counterstaining is not recommended, due to overstaining of the organism. The different parts, head, neck, and tail, with their components, can be readily distinguished.

Figs 1 to 4 show the result obtained with this method. It can also be used for staining other microorganisms such as gonococci, pneumococci, etc.



FIG. 2.—Spirochetes from the oral cavity

The success of the above method can be accounted for by either chemical or physical theories of staining. From the results obtained, it would appear that there exist differences in the isoelectric points of the amphoteric material constituting the blood cells and that of the spirilla. As is well known such ampholytic matter will "take" a basic dye if the hydrogen ion concentration of the staining solution is below its isoelectric point, and an acid dye if above that point. It is not difficult to imagine a difference in staining performance if to two such cellular elements the same dye be added. Should the amphoteric constituents of the spirilla possess a lower isoelectric point than those of the blood cells, the former will more strongly attract the basic dye present in the stain. Such appears to be the case.

*This is the color observed when artificial light is employed. However when the source of light is natural i.e. daylight the organisms appear to be stained a deep blue in contrast to the other elements present which stain a light blue.

On the other hand, the simple principle of mordanting can also be applied here. The constituents of the smear may adsorb the added zirconyl chloride solution to different degrees. With subsequent addition of citric acid, the amounts of basic zirconium citrate formed within the blood cells and the or-

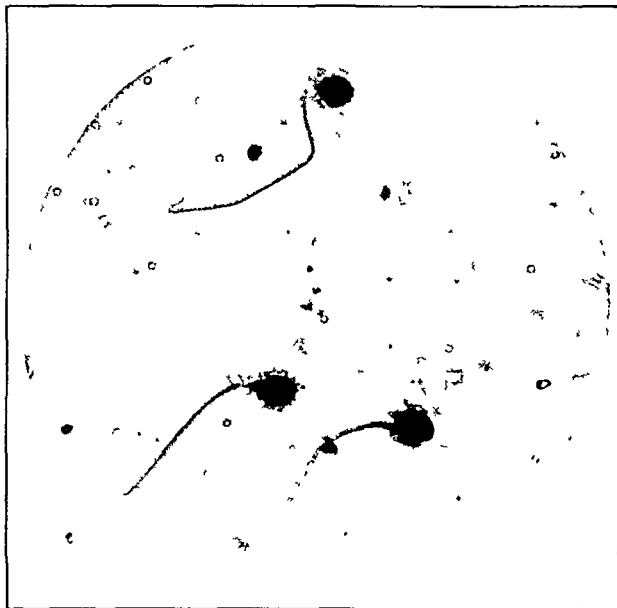


Fig. 3—Human spermatozoa obtained from vaginal secretion.

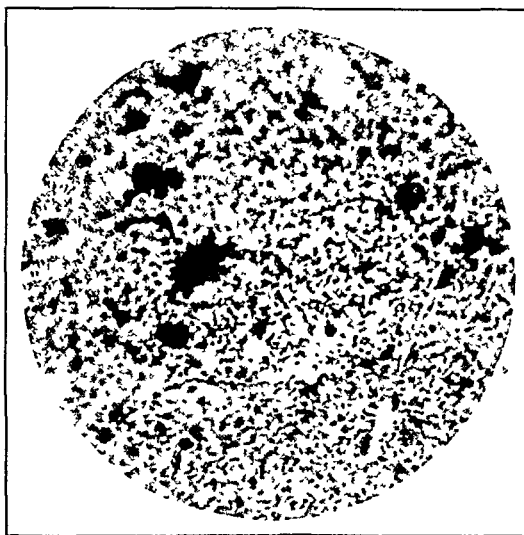


Fig. 4.—*Spirocheta pallida* from human chancre.

ganisms will be different. That such basic citrates are formed is easily demonstrated and their physical nature augurs well for the adsorption of staining materials. The constituent which more easily adsorbs the zirconyl chloride solution will contain more basic citrate and be more deeply stained.

Other salts such as the chlorides of aluminum, bismuth, cadmium, magnesium, mercury, thorium, uranium, copper, and tin were tried in the place of zirconyl chloride. While aluminum and thorium chlorides gave fair results, neither these nor the others were as satisfactory as the zirconium salt.

SUMMARY

1 A rapid method for staining spirilla, spirochetes, and spermatozoa is presented.

2 The organisms are found to stain a deep blue purple, easily identified from surrounding material.

3 Aluminum, bismuth, cadmium, magnesium, mercury, thorium, uranium, copper, and tin chlorides when individually substituted for zirconyl chloride in this method did not give as satisfactory a result.

4 The mechanism of the method is explained on a chemico-physical basis.

52 WEST SEVENTH FOURTH STREET

THE INFLUENCE OF PHYSIOLOGIC SALINES IN COMPLEMENT FIXATION REACTIONS*

J. E. FABER, JR., M.S., AND L. A. BLACK, PH.D., COLLEGE PARK, MD

IN A SERIES of experiments preliminary to a standardization of methods for a study of various factors influencing complement, it was found that titrations of the same hemolysin varied greatly. Various suggestions have been made in the literature regarding the possible effects of saline, and since this seemed to be the cause of the present variations in hemolysin titration, a series of experiments were conducted on salines prepared in various ways.

In a study of the precise titration of complement, Brooks¹ recommended a solution similar to Ringer Tyrode's solution containing 8 gm of sodium chloride, 0.2 gm of potassium chloride, 0.2 gm of calcium chloride, 1 gm of sodium bicarbonate, and 0.05 gm of monobasic sodium phosphate, made up in a liter of distilled water. This physiologically balanced solution was used in place of physiologic saline as a diluent.

The consideration of hydrogen ion concentration seemed very important to Mason and Sanford² and they urged the use in serology of a constant physiologic salt solution. Their buffered solution was prepared by adding 7 gm of sodium chloride, 17 gm of secondary sodium phosphate, and 0.2 gm of primary potassium phosphate to each liter of double distilled water. This gave a solution with a pH of 7.4 to 7.8. They stated that the optimum hydrogen ion concentration for amboceptor titration was between pH 7.0 to 8.4. Boiling and the addition of carbon dioxide changed the concentration of hydrogen ions in distilled water too easily, while tap water did not change as greatly because of the natural buffers present in such water.

*From the Department of Bacteriology, University of Maryland, College Park.
Received for publication August 10, 1930.

The work of Rockwood³ indicated that the effective range of acidity for biologic hemolysis was between the pH of 6.0 to 8.3 with a broad optimum near neutrality. Acid would partly destroy the amboceptor and complement. Mason and Sanford's buffered salt solution, although stabilizing a variable factor in the Wassermann test, did not buffer the final mixture to neutrality since except in extreme cases the buffering value of the cells is enough to accomplish this.

Kellogg and Wells⁴ did not consider the reaction of saline solutions of any great importance. Salines prepared with tap and distilled water of the same pH gave a low titration with distilled water and a very high one with tap water, which they considered was due to the presence of magnesium in the tap water. By adding 0.1 gm. of magnesium chloride or sulphate to a liter of physiologic saline solution the specific lysis of red cells was increased giving higher complement and amboceptor titers.

Kolmer⁵ states that sterilization of saline in an Arnold for an hour (for 1,000 c.c. or a shorter time for a smaller bulk) is preferred to autoclaving because of the concentration of salt in the latter due to the additional loss of water. Kolmer⁶ also recommends the use of 8.5 gm. of chemically pure sodium chloride as the optimum amount of salt. He mentions that the pH of the saline is of practical importance but not the sole factor in selecting a good saline. According to Kolmer the buffered solution of Mason and Sanford is somewhat hemolytic when made up in distilled water and is not as satisfactory as when prepared with Philadelphia tap water, although the range of pH for both was 7.3 to 7.4. He is of the opinion that "the natural buffers of tap water are likely to be more satisfactory in relation to serum hemolysis than artificially buffered distilled water." If saline prepared of distilled or tap water should be unsatisfactory Kolmer recommended that 0.1 gm. of magnesium sulphate or chloride be added to each liter in accordance with Kellogg and Wells.⁴

Recent books on laboratory methods usually recommend distilled water for physiologic saline. Wadsworth⁷ suggests that physiologic salt solution containing 0.85 per cent sodium chloride be used for preparing suspensions of red cells, and for making all dilutions. The required quantity of distilled water is added to the salt, dissolved, filtered through paper, dispensed and sterilized at 121° C. for twenty or thirty minutes, depending on the bulk. It was unnecessary to compensate for small losses of water during sterilization, which usually resulted in a salt solution of about 0.88 per cent. According to Kolmer and Boerner,⁸ one liter of tap or distilled water should be added to 8.5 gm. of dry chemically pure sodium chloride, filtered through paper into a flask fitted with a gauze-covered cotton stopper and sterilized in an Arnold for an hour.

Gradwohl⁹ recommends the use of 8.5 gm. of sodium chloride per 1,000 c.c. of distilled water. This solution is filtered through paper and 500 c.c. brown bottles are filled about two-thirds full, plugged with nonabsorbent cotton, which is then covered with paper and the bottles are then sterilized in the autoclave for thirty minutes at 15 pounds' pressure. He states that fresh saline should be made once or twice a week, and if not stored in brown bottles, should be placed in a dark cabinet.

In the standard procedure of the A P II A for the complement fixation test¹⁰ it is only stated that "the diluent for all reagents should be a salt solution containing chemically pure components isotonic for red cells"

EXPERIMENTAL

Hemolysin Titration—The hemolysin used in all of these experiments was of the same lot prepared by the Difco Laboratories and stated to have a titer of 1 5,000 when used with 0.5 cc of 2 per cent corpuscles and 0.3 cc of 1:30 complement. Complement was secured by pooling the blood of healthy guinea pigs whose titer was already known to be satisfactory, allowing this to stand at room temperature for one hour, refrigerated for twenty-four hours and centrifuged if necessary. The complement was used the same day (except in the last complement experiment when it was used the second day). The corpuscles were obtained from a ewe, defibrinated with glass beads, washed three times or more for ten minutes each at 2,200 r.p.m., packed by centrifuging for twenty minutes, made up to 50 per cent suspension, refrigerated, and used for not longer than two days.

Saline solutions were prepared with 0.85 per cent sodium chloride made up in various waters, and of these some were used unheated, some were placed in flowing steam for thirty minutes, and others autoclaved for thirty minutes. Determinations were made of the pH of these solutions just after use, using a quinhydrone pH indicator at first and later a colorimetric method, using standard acids and solutions that had recently been checked.

A modified Ringer's solution was used in the first two experiments, consisting of 8.0 gm NaCl, 0.14 gm KCl, 0.12 gm CaCl₂, and 0.20 gm NaHCO₃. The various salines prepared were used in each case for dilution of the 1:100 hemolysin and for bringing up the volume. A tap water saline which had been steamed was used for making the first 1:100 dilution of hemolysin and also for diluting the complement and corpuscles.

In the second two experiments a phosphate buffered saline like Mason and Sanford's⁹ was used, except that only single distilled water was used. Another tap water was secured from Washington, D. C., since their tap water is substituted generally there for distilled water for use in batteries, and is of some what different character than other tap water available. The various salines prepared were used in each case for the entire dilution of glycerinated hemolysin and for bringing up the volume. An autoclaved tap water was used in these last two experiments for diluting the complement and corpuscles. In Experiment IV the saline solutions used were those used the preceding day in Experiment III.

In Protocol 1 the arrangement used for each titration of hemolysin with each saline is shown (except additional intermediate dilutions of 1:500-5:500 which were made in the last experiment). The results obtained are tabulated in Table I.

The results of the first experiment in *hemolysin titration* showed a decided increase in titer by using saline prepared from tap water (1:3 or 4:000) compared with saline with distilled water (1:1,000). An increased titer was also

The work of Rockwood³ indicated that the effective range of acidity for biologic hemolysis was between the pH of 6.0 to 8.3 with a broad optimum near neutrality. Acid would partly destroy the amboceptor and complement. Mason and Sanford's buffered salt solution, although stabilizing a variable factor in the Wassermann test, did not buffer the final mixture to neutrality since except in extreme cases the buffering value of the cells is enough to accomplish this.

Kellogg and Wells⁴ did not consider the reaction of saline solutions of any great importance. Salines prepared with tap and distilled water of the same pH gave a low titration with distilled water and a very high one with tap water, which they considered was due to the presence of magnesium in the tap water. By adding 0.1 gm. of magnesium chloride or sulphate to a liter of physiologic saline solution the specific lysis of red cells was increased giving higher complement and amboceptor titers.

Kolmer⁵ states that sterilization of saline in an Arnold for an hour (for 1,000 c.c. or a shorter time for a smaller bulk) is preferred to autoclaving because of the concentration of salt in the latter due to the additional loss of water. Kolmer⁶ also recommends the use of 8.5 gm. of chemically pure sodium chloride as the optimum amount of salt. He mentions that the pH of the saline is of practical importance but not the sole factor in selecting a good saline. According to Kolmer the buffered solution of Mason and Sanford is somewhat hemolytic when made up in distilled water and is not as satisfactory as when prepared with Philadelphia tap water, although the range of pH for both was 7.3 to 7.4. He is of the opinion that "the natural buffers of tap water are likely to be more satisfactory in relation to serum hemolysis than artificially buffered distilled water." If saline prepared of distilled or tap water should be unsatisfactory Kolmer recommended that 0.1 gm. of magnesium sulphate or chloride be added to each liter in accordance with Kellogg and Wells.⁴

Recent books on laboratory methods usually recommend distilled water for physiologic saline. Wadsworth⁷ suggests that physiologic salt solution containing 0.85 per cent sodium chloride be used for preparing suspensions of red cells, and for making all dilutions. The required quantity of distilled water is added to the salt, dissolved, filtered through paper, dispensed and sterilized at 121° C. for twenty or thirty minutes, depending on the bulk. It was unnecessary to compensate for small losses of water during sterilization, which usually resulted in a salt solution of about 0.88 per cent. According to Kolmer and Boerner,⁸ one liter of tap or distilled water should be added to 8.5 gm. of dry chemically pure sodium chloride, filtered through paper into a flask fitted with a gauze-covered cotton stopper and sterilized in an Arnold for an hour.

Gradwohl⁹ recommends the use of 8.5 gm. of sodium chloride per 1,000 c.c. of distilled water. This solution is filtered through paper and 500 c.c. brown bottles are filled about two-thirds full, plugged with nonabsorbent cotton, which is then covered with paper and the bottles are then sterilized in the autoclave for thirty minutes at 15 pounds' pressure. He states that fresh saline should be made once or twice a week, and if not stored in brown bottles, should be placed in a dark cabinet.

phates considerably higher in titer and but little below the tap water, and highest of all was the other tap water used now for the first time. Experiment IV was performed with the same reagents used the preceding day in Experiment III but with a series of intermediate dilutions to allow a more accurate record of titer. A higher titer was usually obtained with autoclaved salines than with unheated salines, the salines heated in flowing steam sometimes being of the same titer as autoclaved salines and sometimes lower, and sometimes as low as the unheated salines. In all cases the pH of the same salines heated or unheated were quite close and there was again a correlation of pH with the titers obtained.

COMPLEMENT TITRATION

Since the results had indicated the influence of the water used in preparing saline upon hemolysin titration, a series of experiments were planned to check these effects upon complement titration.

The reagents used were prepared as indicated under hemolysin titration. A solution similar to Brooks' Ringer Tyrode's solution was also prepared. This formed such a precipitate with tap or distilled water that the solution was unfit for use because it obscured the results.

In Protocol 2 the arrangement used for each titration of complement for each saline is shown. In the experiments themselves the various salines were used for only *one reagent* in each test, the other reagents in each case being prepared with tap water; saline heated in flowing steam for 30 minutes, which was also used for preparing the 1:100 dilution of hemolysin. The results obtained are tabulated in Table II.

A further series of experiments were conducted in which the varying salines were used for *each reagent* in the test, no other saline being used in any place with the exception of the preliminary washing of the blood corpuscles and the preparation to a 50 per cent suspension. In addition, the effect of the addition of magnesium chloride was tested with one distilled and one tap water. The results are presented in Table III.

In the three trials of *complement titration* in which the volume allowed to make up with varying salines (the amount varying from 17 to 19 ml) distilled water was poorer than tap in one, about the same in one, and better in one trial. The addition of phosphates to distilled water improved the titer under these conditions. The improvement in titer due to heating was striking in these experiments, the heated being better in four, the same in one and in one case the autoclaved was slightly poorer than the unheated.

When the *corpuscles* alone were diluted with the various salines being used in each tube, the distilled water gave about the same titer as tap water and the addition of phosphates improved the titer in one series but not in the other series used. However, there was a noticeable uniformity in titer of steamed over unheated salines and of autoclaved over unheated salines. It would seem that the increased evaporation of water and accompanying concentration of salts due to heating the saline did affect the red cells to an extent that the complement titer was correspondingly improved.

indicated in the salines that had been placed in flowing steam for thirty minutes compared with those unheated. The pH of the solutions also varied with the titer with the unbuffered salines, whereas with a modified Ringer's solution there was no correlation of pH and hemolysin titer. Ringer's solution steamed was of equal titer as tap water steamed but unheated Ringer's solution gave a very low titer corresponding to that with distilled water.

Repetition of this experiment after two days with freshly prepared salines gave comparable results as shown by Experiment II. In this case the titer with tap water was not as high as with Ringer's solution (steamed) and likewise the pH of the tap water was not as high as in the preceding experiment.

Other experiments were then conducted on complement titrations but after a month, Experiments III and IV were performed, discontinuing Ringer's solution and adding a phosphate buffered solution (Mason and Sanford's) and another tap water, and including an autoclaved saline in each experiment. Distilled water saline was again lowest in titer, distilled water buffered with phos-

PROTOCOL 1. TITRATION OF HEMOLYSIN

TUBE NO.	A	B	C	D	E	F	G
Diluted hemolysin c.c.	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Complement (1:10) c.c.	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Corpuscles (2%) c.c.	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Saline c.c.	1.9	1.9	1.9	1.9	1.9	1.9	1.9

Incubate at 37° C. for One Hour

RESULTS							
Hemolysin Dilution 1	1,000	2,000	3,000	4,000	5,000	6,000	8,000

TABLE I

HEMOLYSIN TITRATION WITH VARIOUS SALINES

SALINE*		I TITER 1 : pH		II TITER 1 : pH		III TITER 1 : pH†		IV TITER 1 : pH†	
Distilled									
	DU	1000	5.6	1000-	5.9	2000	6.2	1500	6.4
	DS	1000+	6.1	1000+	6.0	2000	6.3	2000	6.4
	DA					2000+	6.3	2000	6.4
Tap									
	TU	3000	7.1	2000-	7.0	3000	7.4	2500	7.4
	TS	4000+	7.3	2000+	7.0	3000	7.4	3000	7.4
	TA					4000+	7.6	4000	7.4
Tap Ringer's									
	TRU	1000	7.9	1000-	7.9				
	TRS	4000	7.8	4000	7.8				
Distilled phosphates									
	DPU					3000	7.1	3000	7.1
	DPS					3000	7.3	3000	7.3
	DPA					3000	7.3	3500	7.3
Tap Washington									
	TWU					4000+	7.8	4500	8.0
	TWS					4000+	8.0	5000	8.0
	TWA					4000+	8.2	5000	8.2

*Complement and corpuscles were diluted with tap water saline, steamed in I, II and autoclaved in III, IV.

D, distilled

T, tap

R, Ringer's

P, phosphates

W, Washington

†Colorimetric pH.

U, unheated

S, steam (flowing)

A, autoclaved

- not quite complete

+nearly complete in next higher dilution

chamber With the base of the counting chamber resting in iced water, fifteen minutes is allowed for settling and the count is made under high power The platelets in eighty small squares are counted and since this represents one fiftieth of a cubic millimeter and the dilution is 1:10, the count is multiplied by 500 to obtain the number of platelets in a cubic millimeter of plasma In cases of thrombocytopenia, no dilution is necessary and after mixture with the heparin the plasma may be run directly into the chilled counting chamber Counting eighty small squares, the result is multiplied by fifty to obtain the number of platelets per cubic millimeter Heparin inhibits the breaking down of platelets by glass provided a low temperature is maintained Other diluents such as sodium citrate or 14 per cent magnesium sulphate might be used instead of the heparin and saline, but when these are used in a 1:10 dilution the platelets showed a tendency to clump Kristensen's fluid is satisfactory when fresh, but it deteriorates rapidly The platelet counts obtained by the method described must be considered plasma platelet counts and hematocrit readings are necessary in order to interpret them in terms of counts for the whole blood Inasmuch as the platelets rise with the plasma and are evenly distributed, the following proportion holds the platelet count for the whole blood is to the plasma platelet count as the plasma volume is to the blood volume, in other words, the plasma platelet count multiplied by the plasma percentage gives the platelet count for the whole blood

The plasma is now transferred to two 8 by 75 mm paraffined tubes One tube containing about 0.5 cc (15 drops) taken from the center of the plasma layer is set aside in ice for a determination of the coagulation time of the whole platelet containing plasma, designated as plasma A The other containing 1.0 to 2.0 cc of plasma is suspended in a Pyrex bottle filled with ice which fits in the large centrifuge cup The plasma is then centrifuged for one and one half hours at 10,000 r.p.m. With an ordinary room temperature it is necessary to repack with ice every half hour During this long centrifuging, all intact platelets are thrown to the bottom of the tube where they may be seen as a grayish mass The supernatant plasma is platelet free but may contain products of platelet disintegration Platelet free plasma is designated as plasma B An amount of plasma B equal to the amount of plasma A is transferred to a similar paraffined tube for determination of coagulation time Likewise an equal amount of plasma is left in the bottom of the tube containing the platelets which have been thrown down by the long centrifuging for determination of the coagulation time of a platelet rich plasma, plasma C For determination of coagulation times, the tubes containing plasma A, B, and C are transferred from ice to a water bath kept at 25° C When a solid clot is formed the fibrin is removed by twirling a wooden applicator stick in the clot, squeezing out all the remaining fluid If a clot reforms, the fibrin is again removed In platelet free plasma, clotting continued to occur throughout a long period, often thirty minutes In a platelet rich plasma, it is usually complete in four to six minutes In the tables the two figures represent the beginning and the completion of coagulation

With this procedure we have for consideration a plasma platelet count, the coagulation time of natural plasma with its full number of platelets, the coagu-

lation time of platelet-free plasma and the coagulation time of platelet-rich plasma. If any difficulty is encountered in getting the blood so that there is the slightest addition of tissue fluid or cerebrospinal fluid, the results are unreliable. This can usually be detected when the platelet count is made. It will be lower than anticipated and the platelets show clumping and variation in size. A coagulation time for plasma A of under ten minutes is also strong evidence that there has been faulty technic. If the procedure is carried through in these instances to the long centrifuging, the plasma usually coagulates during the time of centrifuging or rapidly after removal from ice to the water-bath. In all studies of coagulation factors based upon timing, one should know whether or not there has been addition of tissue fluid to the blood. This method of checking is far from perfect but when anticoagulants are used, no check at all is possible.

RESULTS

Normal adults show a plasma platelet count varying between 250,000 and 500,000. The coagulation time for plasma A ranges between ten and thirty minutes, for plasma B from twenty-five to one hundred minutes, and for plasma C from five to fifteen minutes.

The first deviation from these standards is shown in cases with hemorrhagic symptoms, but with normal or high platelet counts (see Table I). Since these

TABLE I

REMARKS	PLASMA PLATELET COUNT	COAGULATION TIME IN MINUTES		
		PLASMA A	PLASMA B	PLASMA C
Normal	250,000-500,000	10-30	25-100	5-15
Mr. R., petechiae, fever; died with ulcerative colitis (rapid sedimentation)	825,000	22-25	120-138	8-11
M. McH., boy, 8 years following period of bleeding from intestine	550,000	32-34	240-360	17-21
J. McE., boy, 7 years, petechiae, endocarditis	470,000	20-25	115-140	15-17

cases all had normal or high platelet counts, the delayed coagulation would seem necessarily to be due to an increased platelet resistance. No studies have as yet been made on patients with hemophilia.

Cases of thrombocytopenia divide themselves according to this method of study into two distinct groups. The first shows prolonged coagulation times for all plasmas which is sometimes very great for the platelet-free plasma (see Table II). The second group shows a wide variation in the coagulation time of plasma A, but characteristically yields a platelet-free plasma which has a coagulation time of about the same length or even less than that of the whole plasma (see Table III).

In the first group we have two cases of acute leucemia in children. Platelet counts taken without centrifuging the blood, because of the rapid development of a plasma layer, were in the neighborhood of 30,000 for one and 107,750 for the other. Plasma A coagulated in thirty-eight to forty-five minutes, plasma B in from seventy to 180 minutes, and plasma C in from twelve to twenty-five

minutes. A third case, E C, aged six years, showing a single episode of purpura with no recurrence as far as is known, had a platelet count of 46 000, a coagulation time for plasma A of twenty minutes, for plasma B of 430 minutes, and for plasma C of fifteen minutes. The subsequent platelet counts have been 400,000, 475,000, and 550,000 and the coagulation times for all plasma within the normal range. A fifth case, Shirley, showing marked purpuric manifestations of several weeks' duration, had a platelet count of 25,000, a coagulation time for plasma A of forty minutes, for plasma B of 180 minutes, and for plasma C of twenty min-

TABLE II

REMARKS	PLASMA PLATELET COUNT	COAGULATION TIME IN MINUTES		
		PLASMA A	PLASMA B	PLASMA C
L A, boy, leucemia, rapid sedimentation time, hematocrit 15 per cent	4,500	0 45	33 70	20 25
Day following transfusion	23 500	0 45	43 100	15 5
L F, boy, leucemia, rapid sedimentation time	107,750	30 8	1 8	10 12
Lois, colored girl, single purpuric episode, rapid sedimentation time, hematocrit 20 per cent	2 500	5 50	90 200	12 20
E C, boy, single purpuric episode	46,000	19 21	70 4 0	1 15
Question of recurrence, few bruises 1 month later	400 000	14 17	35 45	9
3 days later, recovered	475 000	10	22 30	5
14 months later, no recurrences	550 000	13 18	50 60	8 10
Shirley, girl, purpuric symptoms 23 weeks' duration. Died pulmonary hemorrhage. Question leucemic leukemia	25 000	0 40	180	20

TABLE III

REMARKS	PLASMA PLATELET COUNT	COAGULATION TIME IN MINUTES		
		PLASMA A	PLASMA B	PLASMA C
H B, girl, 8 years, numerous petechiae, long bleeding time	10 000	180	120	21 11
L W, boy, 1 year, repeated bleeding and petechiae many years	1 250	60	15	15
1 day later	2 250	40 50	0 5	15
Day following transfusion	4 000	22 21	17 23	6 11

utes. The child died of pulmonary hemorrhage. Histologic study of the bone marrow showed degeneration of the megakaryocytes with a leucocytic reaction raising the question of an aleucemic leukemia.

In the second group we have a girl H B, aged eight years, who has shown petechiae and hemorrhages of the mucous membranes over a long period of time. Bleeding of the gums continued for four days following the extraction of decayed teeth. Her platelet count was 10,000, the coagulation time for plasma A 180 minutes, for plasma B 120 minutes, and for plasma C twenty seven minutes. A

boy, E. W., aged thirteen years, has had numerous episodes of hemorrhage into the skin and from the mucous membranes. On admission, his platelet count was 1,250. Plasma A required sixty minutes for coagulation, plasma B forty-five minutes, and plasma C fifteen minutes. The next day the platelets were 2,250 and the coagulation time for plasma A was fifty minutes, for plasma B thirty-five minutes, and for plasma C fifteen minutes. A transfusion was given on the day following admission and the following day the platelets were 4,000, the coagulation time for plasma A thirty-one minutes, for plasma B twenty-three minutes, and for plasma C eleven minutes.

The findings in these cases of thrombocytopenia suggest that in the first group we are dealing with platelets which, though reduced in number, are fairly normal in character. They undergo relatively little disintegration during the process of centrifuging them out of the plasma and the coagulation time of plasma A is prolonged in most cases, proportionately to the reduction in total platelet count. There is the possibility that the prolongation of coagulation represents an increased resistance as well as a reduction in numbers. This is particularly indicated in several of the cases by the prolonged coagulation time of the platelet-rich plasma. In the second group, where we are dealing with cases of long-standing purpura, resembling the syndrome described by Werlhof, the platelets do not show a normal resistance. They are broken down during the centrifuging, yielding a platelet-free plasma which coagulates more rapidly than plasma A. Whether this is due to properties inherent in the platelets or to changes produced during the course of their circulation in the blood stream must be the subject of further investigation. It is possible in these cases that the platelets are produced by the bone marrow in normal numbers but that many undergo destruction in the blood stream and that those remaining show a tendency to increased fragility.

CONCLUSIONS

1. A method of estimating platelets quantitatively and qualitatively in their own plasma is described.

2. It depends upon a consideration of the coagulation times of whole plasma, platelet-free plasma and platelet-rich plasma.

3. By use of chilled, oiled syringes and paraffined tubes, errors due to contact with glass are avoided, and as no anticoagulant is used, it is possible to check fairly accurately whether or not the blood has been contaminated with tissue fluid. If this has occurred, the blood is unsuitable for further study.

4. Among miscellaneous cases with normal or high platelet counts which had presented hemorrhagic symptoms, three were found which had unusually resistant platelets.

5. Cases with thrombocytopenia divided themselves into two groups, one with platelets showing normal or increased resistance, and the other with platelets giving evidence of increased fragility. From these observations, cases in the first group belong to the symptomatic type of purpura and those in the second group to the so-called essential thrombocytopenia type.

REFERENCES

1. Fonio, A.: Plasma Obtained With and Without Platelets, *Ztschr. f. klin. Med.* 119: 687, 1932.

2. Nygaard, K. K.: A Direct Method of Counting Platelets in Ovalated Fluid. Staff Meetings, Mayo Clinic 8: 361, 1933.
3. Cummings, J. N.: A Method of Platelet Estimation, Lancet 1: 1230, 1933.
4. Lampert, H.: Further Results With the Chamber Method of Thrombocyte Counting, Deutsche med. Wchnschr. 57: 2016, 1931.

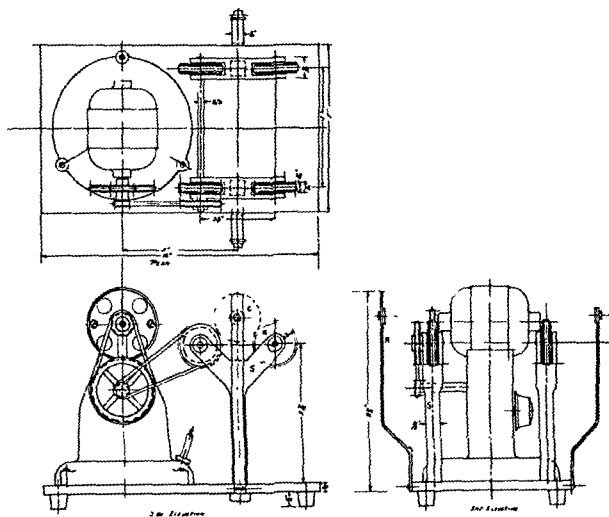
636 CHURCH STREET, EVANSTON

932 N. MICHIGAN AVENUE, CHICAGO

A BALL MILL FOR GRINDING SMALL QUANTITIES OF BACTERIA*

JOHN F. NORTON, JOHN H. DINGLE, AND T. HERBERT SHENSTONE,
KALAMAZOO, MICH.

IN THE course of studies on the antigenic constituents of bacteria, it was necessary to employ a ball mill which would conveniently grind small amounts of bacteria in either wet or dry condition. Such a mill is described here.



Pyrex glass or stainless steel containers, 8 inches long and $1\frac{3}{4}$ to 3 inches in diameter, are employed. These may be closed with rubber stoppers or

*From the Department of Bacteriology, The Upjohn Company.
Received for publication, August 5, 1935.

fitted lids. Approximately 1,000 stainless steel balls,* $\frac{1}{8}$ in. (3.2 mm.) in diameter, are placed in the container with the bacteria. Both container and steel balls are easily sterilized if asepsis is desired. The container (*C*) is placed upon two sets of rubber rollers (*O*), supported by V-shaped standards (*S*), and is held in place by adjustable retainers (*R*) at each end as shown in the accompanying drawing. Base, standards, and retainers are constructed of cold rolled steel. Rollers are of rubber with brass side plates. Power is supplied by a variable speed motor,† and speed is controlled by the motor or by pulleys to obtain a speed of 140 to 250 revolutions per minute. The motor and rollers are connected by heavy rubber belts.

Eighteen to twenty-four hours of grinding is sufficient in most cases to produce a satisfactory disruption of staphylococci, typhoid and pertussis organisms.

DETERMINATION OF FIBRIN BY THE BIURET METHOD‡

JOSEPH FINE, M.D., D.P.H., B.Sc., LONDON, ENG.

IT HAS been shown (Fine, 1935) that the albumin and globulin of serum can be estimated rapidly and satisfactorily by an application of the biuret technic. A successful extension of the method to the proteins of plasma clearly must depend on whether fibrin can likewise be estimated. This was considered probable when it was found that fibrin yields a violet solution with CuSO_4 and NaOH , which in the colorimeter matches perfectly the color yielded by serum proteins.

Methods of Estimating Fibrin in Plasma.—These methods fall into three main groups: the Kjeldahl (Cullen and Van Slyke, 1920); gravimetric (Gram, 1921; Foster and Whipple, 1921); and colorimetric (Wu, 1922). In all these methods the fibrin is first separated from the plasma by recalcification with CaCl_2 , though Howe (1923) in another Kjeldahl method precipitates the fibrinogen with 10.6 per cent Na_2SO_4 . Of the above methods that of Wu was most suitable for rapid clinical purposes, but it is suggested that the biuret technic will now be the method of choice.

Biuret Determination of Fibrin.—This was carried out on a sample of commercial fibrin and on several samples of human plasma, the results being compared with gravimetric determinations. The standard for the biuret method was a 0.24 per cent serum protein solution, as previously described in the method for serum proteins.

Commercial Fibrin: The biuret method was carried out by dissolving a weighed amount in 1 ml. of hot 30 per cent NaOH and making up the cooled solution to 10 ml. with distilled water and 1 ml. of 5 per cent CuSO_4 . The mixture was shaken for ten seconds and the supernatant fluid after centrifuging was compared with the violet solution obtained from the standard. The

*Manufactured by Strom Steel Ball Company, Cicero, Illinois.

†Manufactured by Burgess-Parr Company, Moline, Illinois.

‡From the Laboratory of Chemical Pathology, St. Bartholomew's Hospital.
Received for publication, August 12, 1935.

gravimetric determination was made by heating a weighed amount at 110°C to constant weight and then ashing. The weight of ash + moisture was subtracted from the weight of the sample and the difference expressed as a percentage of the sample.

Plasma Fibrin. The first stage in both the buret and the gravimetric determinations was the recalcification of the oxalated plasma by the method of Cullen and Van Slyke (1920), 2 cc. of plasma being added to 60 cc. of 0.85 per cent NaCl , and 2 cc. of 5 per cent $\text{CaCl}_2 \cdot 6\text{H}_2\text{O}$ (or 2.5 per cent anhydrous CaCl_2), and the clot which formed in one half to one hour collected in the usual way on a glass rod, slipped off and washed in distilled water. From this stage the buret determination was carried out in the same way as for commercial fibrin. In the case of the gravimetric determination the dry weight and the weight of the ash were first obtained. The weight of the ash (CaO) was then multiplied by $\frac{16}{7} = \frac{\text{CaC}_2\text{O}_4}{\text{CaO}}$ and the result subtracted from the dry weight to give the weight of actual fibrin. This calculation, not elsewhere suggested, is clearly necessary since the Ca which presumably is combined as oxalate in the dried fibrin is converted into oxide during ashing. The calculation is not required for commercial fibrin owing to the small amount of ash present ($\frac{1}{2}$ per cent); the fibrin of recalcified plasma, however, may contain 10 or 20 per cent ash owing to the incorporation in the clot of calcium oxalate derived from interaction between excess CaCl_2 and excess potassium oxalate. Failure to make the above calculation gave grossly high results for the fibrin of normal blood, a consequence theoretically to be expected. When, however, the calculation was made, not only did the resulting gravimetric figures fall within normal limits but they also agreed very closely with the figures given by the buret method.

TABLE I
RESULTS

SAMPLE	PER CENT FIBRIN	
	GRAVIMETRIC	BUURET
Plasma 1	—	0.88
"	0.10	0.78
"	0.75	0.75
Commercial fibrin	90.0	85.0

Technic for Estimation of Plasma Proteins—

1 Total Proteins } The procedure is identical with that elsewhere described for serum proteins (Pine, 1935)

2 Albumin

3 Fibrin. 2 cc. plasma is recalcified by the method of Cullen and Van Slyke (1920) outlined above. The fibrin yielded is dissolved by heating in 1 cc. 30 per cent NaOH in a 15 cc. centrifuge tube, and the estimation is continued as for total proteins.

4 Standards. The standard required for total proteins and albumin is prepared from 5 cc. of a 0.24 per cent serum protein solution. Unless fibrin value is expected a second standard should be prepared of the above solution.

Calculation: If the unknown is set at 20 mm., and the readings of the standard for total protein, albumin, and fibrin are X, Y, Z, respectively, then

Total protein	= 0.3X per cent
Albumin	= 0.24Y per cent
Fibrin	= 0.03Z per cent, using 5 c.c. standard or 0.015Z per cent, using 2.5 c.c. standard
Globulin	= [0.3X - 0.24Y - 0.03Z] per cent or [0.3X - 0.24Y - 0.015Z] per cent

SUMMARY

The estimation of fibrin by the biuret method, using serum protein as standard, gives results closely agreeing with those by the gravimetric method. A technic is outlined for the estimation of plasma proteins by the biuret method, which is considered the method of choice for clinical purposes.

I am indebted to Dr. G. A. Harrison for permission to carry out the above investigation in his laboratory.

REFERENCES

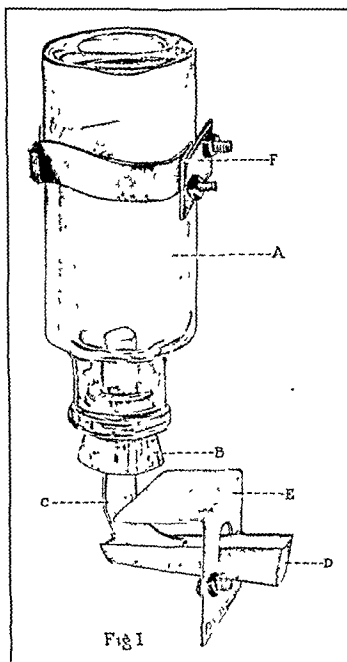
1. Cullen, G. E., and Van Slyke, D. D.: Determination of the Fibrin, Globulin and Albumin Nitrogen of Blood Plasma, *J. Biol. Chem.* 41: 587, 1920.
2. Fine, J.: The Biuret Method of Estimating Albumin and Globulin in Serum and Urine, *Biochem. J.* 29: 799, 1935.
3. Foster, D. P., and Whipple, G. H.: Third Section of Blood Fibrin Studies: Fibrin Values Influenced by Transfusion, Haemorrhage, Plasma Depletion, and Blood Pressure Changes, *Am. J. Physiol.* 58: 393, 1921.
4. Gram, C. H.: A New Method for the Determination of the Fibrin Percentage in Blood and Plasma, *J. Biol. Chem.* 49: 279, 1921.
5. Howe, Paul E.: The Determination of Fibrinogen by Precipitation with Sodium Sulphate Compared with the Precipitation of Fibrin by the Addition of Calcium Chloride, *J. Biol. Chem.* 57: 235, 1923.
6. Wu, Hsien: A New Colorimetric Method for the Determination of Plasma Proteins, *J. Biol. Chem.* 51: 33, 1922.

A SATISFACTORY DRINKING FOUNTAIN FOR CAGED ANIMALS*

GEORGE WALKER, M.D., BALTIMORE, MD.

THE accompanying cut shows an apparatus for supplying fresh, clean water to animals in cages. The principle on which it works is an old one.

It consists of an ordinary six-ounce wide-mouthed bottle (Fig. I, A), a perforated cork (Fig. I, B), a curved glass tube (Fig. I, C), a metal water



trough (Fig. I, D) held in place by a metal holder (Fig. I, E), while the bottle, in turn, is held on the outside of the cage by a spring clamp (Fig. I, F), which is fastened to the exterior of the cage by two screws. The metal holder which supports the trough and tube is attached to the cage by two screws and the end of the trough extends through a hole cut in the wire with a pair of clippers. This

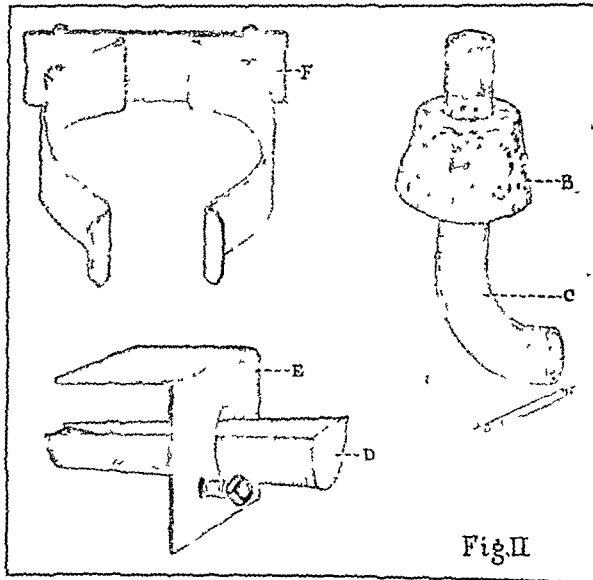
*Received for publication, August 6, 1935.

hole is made sufficiently large to accommodate the trough, but not large enough for small animals to get through. The end of the trough which extends into the cage is deeper than the end on which the tube rests. This arrangement insures a proper flow of water.

As the animal drinks, the water in the trough sinks below the level of the upper edge of the glass tube and allows air to bubble up and water in turn to run down.

The bottle is easily detached from the cage and may be washed and filled daily.

A larger trough (Fig. II) is made for animals such as rabbits, guinea pigs, and fowls.



The advantages are:

1. This apparatus insures clean fresh water.
2. The trough is placed about two inches from the floor of the cage thus protected from becoming contaminated with dirt and trash.
3. The animal drinks with ease.
4. The bottle is always visible so that one can tell at a glance what cage is being supplied with water.
5. Water can be supplied without opening the cage.

This complete drinking outfit can be made with little trouble or can be purchased from George H. Wahlmann, 18 North Greene Street, Baltimore, Maryland.

1 EAST CENTER STREET

MODIFICATION OF FOLIN AND WU'S METHOD FOR SUGAR DETERMINATION*

MILVILF SAHYUN, PH D, DETROIT, MICH

THE method for blood sugar determination described in this paper is a modification of Somogyi's¹ and Folin and Wu's.² Somogyi was first to suggest the use of 10 per cent sodium tungstate and 7 per cent copper sulphate solutions for the precipitation of blood proteins. When these reagents were tried on tissue proteins³ the concentration of copper sulphate was found inadequate, for the filtrates so obtained were not completely protein free. Consequently, the concentration of copper sulphate was increased to 10 per cent.

In an earlier paper by the author⁴ it was pointed out that the addition of 0.25 M sodium sulphate to the alkaline copper sulphate reagent of Folin and Wu increased the stability of the final blue color. This reagent was adopted.

The various reagents required are as follows:

REAGENTS

Ten Per Cent Copper Sulphate—Dissolve 100 gm of copper sulphate crystal in 900 cc of distilled water. Heat to boiling until the copper sulphate is dissolved overnight, filter, and dilute to one liter.

Ten Per Cent Sodium Tungstate—Prepare a 10 per cent solution in the usual manner.

Alkaline Copper Sulphate—Dissolve 50 gm of anhydrous sodium carbonate in 500 cc of water containing 0.25 M sodium sulphate. Add 7.5 gm of tartaric acid until the latter is dissolved. Add 4.5 gm of pure copper sulphate. When the latter is dissolved dilute to one liter. Allow the mixture to stand 1 hour. The filtrate must be perfectly clear.

Phosphomolybdic Acid—Introduce into a two liter beaker 100 gm of phosphoric acid (ammonia free) and 10 gm of sodium tungstate. Add 800 cc of sodium hydroxide. Boil vigorously for thirty minutes. Cool. Add about 100 cc of concentrated phosphoric acid (85 per cent). Dilute to one liter.

Note—Special care should be taken in thoroughly cleaning the glassware used in the preparation of these reagents.

Standard Sugar Solution—Prepare a stock solution of 1 per cent of the purest quality available in a saturated solution of benzoic acid. This stock solution keeps indefinitely.

One Tenth Milligram of Glucose Standard—Dilute 1 cc of the 1 per cent stock glucose solution into a 500 cc volumetric flask with a solution of benzoic acid. One cubic centimeter of this solution contains 0.1 mg of glucose.

If necessary 0.05 and 0.2 mg glucose standards may be prepared.

Benzoic Acid—Transfer 29 gm of benzoic acid to a 500 cc volumetric flask. Add distilled water. Heat to boiling until the acid is dissolved. Use this benzoic acid solution for the preparation of the standard glucose solutions.

Glucose standards prepared in the usual manner.

*From the Biochemical Laboratory,
Received for publication

Precipitation of Blood Proteins.—Transfer 1 c.c. of blood into a flask containing 7 c.c. of distilled water. Add 1 c.c. of 10 per cent sodium tungstate and mix. Add 1 c.c. of the 10 per cent copper sulphate and shake vigorously. The 10 per cent copper reagent must always be added last. Allow the mixture to stand for ten minutes before filtration.

METHOD

Pipette 2 c.c. of the protein-free filtrate into a 25 c.c. Folin sugar tube and to another tube pipette 2 c.c. of the glucose standard (0.1 mg. per 1 c.c.). In the event that blood sugar is low, use the substandard of 0.05 mg. simultaneously. To each tube add 2 c.c. of the special alkaline copper sulphate. Transfer

TABLE I
BLOOD SUGAR DETERMINATIONS

SAMPLE	FOLIN AND SVEDBERG METHOD ⁵	DESCRIBED METHOD
	MG. PER 100 C.C. OF BLOOD	MG. PER 100 C.C. OF BLOOD
1	MG. 99	MG. 95
2	110	110
3	89	89
4	74	77
5	111	111
6	106	109
7	97	95
8	85	83
9	148	148

TABLE II
THE ESTIMATION OF BLOOD SUGAR BY BENEDICT'S METHOD AND BY THE
METHOD DESCRIBED

SAMPLE	MG. BLOOD SUGAR PER 100 C.C.	
	BENEDICT'S ⁶	DESCRIBED METHOD
1	MG. 125	MG. 122
2	104	108
3	103	102
4	115	114
5	89	88
6	83	78
7	91	87
8	108	105

TABLE III
DETERMINATION OF BLOOD SUGAR BY FERMENTATION

The blood glucose was subjected to yeast fermentation and the "saccharoids" in the filtrates were determined. The difference between the reducing substances in the unfermented sample of blood and in the same sample after fermentation is considered fermentable sugar.

Reducing and nonreducing substances in blood, mg. per 100 c.c.

SAMPLE	TOTAL REDUCING SUBSTANCES	FERMENTABLE SUGAR	NONFERMENTABLE SUGAR
1	MG. 83	MG. 77	MG. 3
2	121	116	5
3	83	81	2

the tubes to a boiling water-bath and boil for six minutes. Remove and cool for three or four minutes in a water-bath. Add to each 2 c.c. of the phosphomolybdic acid reagent and five minutes later dilute to the 25 c.c. mark. Mix by inverting the tubes 3 times. Compare colors.

CONCLUSION

The main feature of the method described is the stability of the blue color developed upon the addition of the phosphomolybdic acid reagent. This improvement permits the simultaneous determination of a large number of samples. Furthermore, it is believed that the estimation of blood sugar by this method is close to the true sugar content of blood. This assumption is based not only on a comparative determination of the blood sugar by such standard methods as Benedict's and Folin and Svedberg's methods, but also by the fermentation method described by Somogyi.⁷

REFERENCES

1. Somogyi, M.: The Use of Copper and Iron Salts for the Deproteinization of Blood, *J. Biol. Chem.* 90: 725, 1931.
2. Folin, O., and Wu, H.: System of Blood Analysis, *J. Biol. Chem.* 41: 367, 1920.
3. Sahyun, M.: On the Carbohydrate of Muscle, *J. Biol. Chem.* 94: 253, 1931.
4. Sahyun, M.: Determination of Glycogen in Tissues, *J. Biol. Chem.* 93: 227, 1931.
5. Folin, O., and Svedberg, A.: Determination of Sugar in Unlaked Blood, *J. Biol. Chem.* 88: 85, 1930.
6. Benedict, S.: Analysis of Whole Blood, *J. Biol. Chem.* 92: 135, 1931.
7. Somogyi, M.: Notes on Sugar Determinations, *J. Biol. Chem.* 70: 599, 1926.

A RAPID METHOD FOR ROUTINE SERUM PROTEIN DETERMINATION*

RUSSEL O. BOWMAN, PH.D., PROVIDENCE, R. I.

MOST hospital laboratories, where serum protein determinations are done, use the Howe¹ method or some modification of it. Because this method is time-consuming and somewhat technical, there are many hospitals where serum protein determinations are not part of the laboratory routine. There is great need for a rapid, accurate method for serum protein estimation, so that this information may be available to more clinicians as an aid in diagnosis and treatment of renal, cardiac, hepatic, and nutritional disturbances.

In 1920, Denis and Ayer² published a method for determination of cerebrospinal fluid protein. This method can be applied to the determination of serum or plasma proteins, and can also be used, after sodium sulphate separation, to determine the albumin and globulin fractions present. The method has been used at this hospital for over a year with success.

REAGENTS

1. Protein Stock Standard Solution: 20 c.c. of fresh clear human serum are diluted to 200 c.c. in a volumetric flask with 15 per cent sodium chloride solution. Mix and filter. Add a few crystals of thymol and the solution will keep for six months or more in the ice chest.³ Total nitrogen of this filtrate is determined by the micro-Kjeldahl method and nonprotein nitrogen is determined on a sample of the original serum by the Folin-Wu method.⁴ The total nitrogen of the filtrate minus one-tenth of the nonprotein nitrogen of the serum gives protein nitrogen, and protein nitrogen multiplied by 6.25 gives the protein content of the filtrate.

2. Protein Standard Solution: The stock standard is diluted with distilled water so as to contain 30 mg. per 100 c.c. of solution. This standard keeps for one year when preserved with thymol and kept in the ice chest.

3. 5 per cent Sulphosalicylic Acid: Dissolve 50 gm. of C. P. sulphosalicylic acid in distilled water and make up to a liter. Filter. This keeps indefinitely.

4. 22.2 per cent Sodium Sulphate: 111 gm. of anhydrous C. P. Na_2SO_4 are dissolved in distilled water with the aid of heating to 37° C. in a 500 c.c. volumetric flask. The volume is made up to the mark at this temperature. With purified salt, filtering is not necessary. This reagent is kept at 37° C. in the incubator to prevent crystallization of the salt at room temperature.

5. 0.9 per cent Sodium Chloride.

METHOD

Exactly 0.2 c.c. of blood plasma or serum is diluted by adding 39.8 c.c. of normal saline from a buret. If the determination is to be run at once, distilled water may be used instead of the saline.

*From the Laboratories of Rhode Island Hospital.
Received for publication, September 12, 1935.

occasionally triplicate determinations. Check determinations showed better agreement by the sulphosalicylic acid method than by the digestion method, proving that there is less chance for technical error in the present method.

Table II gives a further comparison of the sulphosalicylic acid and gravimetric methods.⁵ These two methods agree quite well and the gravimetric method is considered the most accurate determination of serum protein yet devised.

Because of the relation of serum protein to serum calcium concentration and the necessity of determining calcium, inorganic phosphorus, and total protein on the smallest possible amount of serum, the method has been applied for determination of all three on a sample of 2 c.c. of serum. Calcium and inorganic phosphorus were determined according to Lowenberg and Mattice.⁶ The small amount of supernatant fluid from calcium precipitation which remains after part is used for phosphorus determination, is sufficient for protein estimation. Two-tenths cubic centimeters of this supernatant fluid, which is a 2:5 dilution of the serum, is diluted with 15.8 c.c. of distilled water or saline from a buret so as to make a 1:200 dilution of the serum. This is compared with a standard as given above for total protein estimation.

A series of tests were run using 0.2 per cent ammonium oxalate, 0.9 per cent saline and weak buffer solutions of phosphates of pH 6.8 to 9.0 as diluting fluids before sulphosalicylic acid was added. Results were alike for the different diluents used. When distilled water is used to dilute certain sera or plasmas, especially when diluted and let stand, there is a partial precipitation of globulin. This can be redissolved by adding a few crystals of sodium chloride to the tube and mixing, and without any effect on the final result. When determination is done right after dilution, saline or distilled water may be used as a diluent without affecting the value obtained.

Publication of this article has been delayed for a year because on two different samples it was not possible to check the micro-Kjeldahl and sulphosalicylic acid procedures. In each of these cases the sulphosalicylic acid method gave much lower figures than the digestion method. Lipemia or Bence-Jones proteinemia was not present. Further work to explain the discrepancy was impossible since in each case the sample was obtained shortly before death of the patient. Several more checks have been obtained by the two methods and no other puzzling results have been obtained.

DISCUSSION

It has been shown that oxalate present in oxalated plasma does not affect the determination of protein by this method. Moderate changes in pH of the diluted sample do not change the results. Because of the large dilution factor, any changes present in pathologic bloods will have little or no effect on the final result. The method seems to be applicable to all sera or plasmas and suitable for routine work in a hospital laboratory.

It is interesting to note that the cost to the hospital of the sulphosalicylic acid method is about one-tenth the cost of the digestion method. Total protein determinations can be run at a cost of about five cents each, including labor, materials and chemicals.

The most important source of error in the present method is in measurement of the initial sample for dilution. Any error here is multiplied many times by the dilution factor. The satisfactory results above were obtained by delivering between marks on a 1 c c Egan pipette graduated in 1/100 c c. Other errors to be avoided are sedimentation of the precipitated protein in too concentrated solutions, incomplete mixing of diluted unknowns, and unequal light to the colorimeter cups.

SUMMARY

The Denis Ayer method for quantitative determination of protein in cerebrospinal fluid has been applied to blood serum or plasma protein estimation.

This method, with care, is as accurate as micro Kjeldahl determination with nesslerization, or a gravimetric procedure. It can be used for the determination of albumin and globulin fractions of blood serum or plasma protein.

A method is given for determination of calcium, inorganic phosphorus and total protein on 2 c c of blood serum.

REFERENCES

1. Howe, P. E. The Determination of Proteins in Blood—A Micro Method, *J Biol Chem* 49 109, 1921.
2. Denis, W., and Ayer, J. B. A Method for the Quantitative Determination of Protein in Cerebrospinal Fluid, *Arch Int Med* 26 436, 1920.
3. Ayer, J. B., and Foster, H. D. Quantitative Estimation of the Total Protein in the Cerebrospinal Fluid, *J A M A* 77 365, 1921.
4. Folin, O., and Wu, H. A System of Blood Analysis, *J Biol Chem* 38 81, 1919.
5. Guillaumin, C. O., Wahl, R., and Laurencin, M. L. Sur le dosage des albumines sériques. Comparaison de quelques résultats obtenus par pesée, par azotométrie, et par réfractométrie, *Bull Soc Chim Biol* 11 387, 1929.
6. Lowenberg, C., and Mattee, M. Note on the Determination of Inorganic Phosphate of Serum and Spinal Fluid on the Supernatant Fluid from Calcium Estimation, *J Lab & Clin Med* 15 598, 1930.

The results of the gonococcic complement fixation and Wassermann tests of the blood and synovial fluids were in agreement. Both tests were of distinct aid in the etiologic diagnosis of disease of the joints.

The total cell count of the synovial fluid was increased in all the types of arthritis studied. It was highest in the infected fluids and lowest in the cases of charcot joints and traumatic arthritis. Most noninfected fluids contained less than 40,000 cells per c.mm.

When the synovial fluid was infected with microorganisms, the polymorphonuclear cells were greatly increased, from 86 to 100 per cent, and the lymphocytes, monocytes and clasmatoocytes were few in number. In tuberculosis of the joints, the polymorphonuclear count varied from 46 to 93 per cent, and the lymphocytes and monocytes were increased.

The percentages of lymphocytes, clasmatoocytes and monocytes were always higher in the noninfected fluids than in the infected fluids. The presence of a low cell count with an increase in monocytes, lymphocytes and clasmatoocytes was an indication of a noninfected fluid.

The chemical examination of the fluids yielded no information of diagnostic value in discriminating between infected and noninfected fluids. The nonprotein nitrogen of both the infected and the noninfected synovial fluids was the same as that of the blood. The sugar content varied with the presence of organisms, the number of cells and the level of the sugar in the blood. A low sugar content did not always mean an infected fluid. The total protein value of the synovial fluid was increased in both groups, and indicated only an inflammatory reaction.

Aside from the bacteriologic, cytologic and serologic examinations of the synovial fluid, other tests yielded little information of diagnostic value.

JAUNDICE, Mechanism of, Elton, N. W. Am. J. Clin. Path. 5: 40, 1935.

Application of the ring test modification of the van den Bergh reaction to the study of jaundice reveals many data inconsistent with prevailing classifications and concepts of its nature. The commonly accepted concept of hemolytic jaundice has been found to be especially fallacious. With these newer data a working hypothesis has been constructed to explain the mechanism of jaundice on the basis of the bilirubin excretion mechanism, disturbances of which are operative in producing it. Data from the work of many investigators have been correlated in this analysis.

The liver is the excretory organ for bilirubin. Bilirubin in the mammal is produced chiefly extrahepatically. Being insoluble in aqueous solvents except alkalis, bilirubin enters the blood stream from its sites of origin in a state of colloidal suspension. The normal alkalinity of the blood is insufficient to change its nascent state. The liver, in excreting bilirubin, converts it into a water-soluble salt, a bilirubinate. Since bilirubin is an acidic pigment, the alkaline bile salt of the liver parenchyma is believed to be the agent which neutralizes the pigment sufficiently to form the salt.

The structural design of the liver lobule, the known affinity of the Kupffer cell for solid particles, and a slight modification of the bile secretion mechanism as conceived by O. S. P. lead to the division of the liver lobule, from the standpoint of bilirubin excretion,

5. major functional zones and an intermediate zone, as follows:

Apical zone, involving the peripheral portion of the lobule, where bilirubin is cups and p. low flow sinusoidal stream by Kupffer cells and passed on by them into 35 and 47 wh. conversion and excretion.

Method.—The intermediate zone midway between the periphery and the center to a water-soluble salt, a bilirubinate, as it passes toward the central portion of the lobule.

between 0.05 and 0.5 " lobule, where bilirubinate is "s into the bile canaliculi,

" view of etiologic factors as follows: (1)

mechanical obstruction of the bile ducts, (2) dynamic elevation of the excretion threshold, (3) adynamic elevation of the excretion threshold, (4) pigment overload, (5) damage to liver parenchyma, (6) patent ductus venosus

TUBERCLE BACILLUS, The Progeny of, Maher, S J Am Rev Tuberc 31 350, 1935

In this paper Maher reports and summarizes the results of his studies on the mutation of tubercle bacilli in laboratory cultures whereby they become cocci and diplococci nonacid fast and in this form produce an acid harmless to themselves but harmful to the bacilli from which they are derived

The paper summarizes these studies since their inception in 1908 and describes the method employed to bring about the transformation so that it may be employed by those interested. It has succeeded with all forms of tubercle bacilli human, bovine and avian and with cultures habituated to growth at room temperature as well as fresh cultures growing only at 37° C

While mutation can be induced in tube cultures as well as in flask cultures, it is more certain and occurs more rapidly in the latter which are therefore more suitable for the purpose

Method—Flask cultures To a culture of tubercle bacilli on glycerin broth (pH 6.4-7.2) add a volume of sterile litmus milk (prepared from skimmed milk) equal to 25 or 30 per cent of the total volume of glycerin broth. In other words, to a culture having a volume of 250 cc from 50 to 60 cc of litmus milk is added. The flask is then stored in the dark at room temperature until the litmus blue color begins to change to pink or white, when it is ready for examination. In the case of young cultures incubation for a few hours at 37° C after addition of the milk will facilitate mutation.

The growth on solid media is scraped off and emulsified in 2 cc of sterile plain broth and the emulsion poured into 250 cc of glycerin broth. Then add 50 cc of sterile litmus milk and incubate as described.

The course of events is somewhat as follows. The first change, apparent usually within 4 or 6 days, is a whitening of the color of the medium in its upper layers. Examination of smears at this time shows, in addition to granular acid fast rods, free red and blue granules and many blue (i.e. nonacid fast) cocci and diplococci. When mutation has been complete and no acid fast forms are found, the cocci and diplococci may be transplanted to other media upon which they grow readily.

Sometimes mutation is slow and even when the acid production is evidenced by the formation of curds and whey in the flask only tiny, nonacid fast rods will be seen in the smears. If these are subcultured and incubated at 37° C, however, transformation into cocci and diplococci is prompt.

The various changes in the appearance of the transmuting organisms is described in the paper in detail.

Whether or not the fact that the transmuted forms which, by virtue of their acid production, produce a condition inhibitory to the growth of tubercle bacilli, may have any practical application to the treatment of tuberculosis remains for future studies to develop.

TUBERCULIN REACTIONS Evaluation of White Blood Cell Picture in Ambulatory Child With Positive, Smith, C H Am J Dis Child 49 318, 1935

In a group of twenty three ambulatory patients with the childhood type of tuberculosis, the white blood cell count was examined periodically with the supravital technic in an endeavor to discover any significant alterations produced by manifest disease.

Since differential percentages vary with age, the results were separated into two groups and compared with similar blood counts of normal children. The age periods extended from infancy to four years and from four to thirteen years.

The peripheral blood of the ambulatory child who was already infected but whose contact with the source of exposure had been broken differed except for minor alterations, in no wise from the normal. In general, a lymphocytic increase, indicative of resistance, constituted the prominent feature.

TISSUE: Butyl Alcohol and Cytological Technic, Zirke, C. Science 80: 481, 1934.

The following mixture is very useful for cleaning slides.

Water	1 part
Xylene	1 part
N-butyl alcohol	1 part
Ethyl alcohol	2 parts

A few drops of n-butyl alcohol added to clouded xylene or alcohol in tissue staining jars will keep the solutions clear and usable.

If the following constitute the original series no precipitate occurs and the solutions remain good for nearly 100 slides.

1. Xylol, 100 per cent.
2. Xylol, 95 per cent, n-butyl alcohol 5 per cent.
3. Absolute ethyl alcohol 90 per cent, n-butyl alcohol 10 per cent.
4. Absolute ethyl alcohol 100 per cent, etc.

PROTEUS X-19, Pleo-Antigenicity of, Welch, H., Mickle, F. L., and Borman, E. K. Am. J. Pub. Health 24: 1157, 1934.

The morphologic and biochemical characteristics of 19 diagnostic *Proteus* X strains and 5 variants are reported in detail. Marked differences in these characteristics were not noted except for the finding of 13 motile strains in the diagnostic group.

Heterologous antigenic components in common with the *Shigella*, *Eberthella*, and *Salmonella* groups were shown in 11 *Proteus* X strains. Although these were not demonstrable by direct agglutination in heterologous sera, they were demonstrated in the antisera produced by them on injection into rabbits or failing this, by their ability to absorb heterologous agglutinins from other *Proteus* sera containing them. Only one of the strains for which sera prepared showed the entire absence of heterologous components.

The heterologous agglutinins present in *Proteus* X sera were shown to be bacterial group agglutinins.

Known variants of *Proteus* X strains were shown to be practically inagglutinable in sera from clinical cases of Rocky Mountain spotted fever and, in addition, certain of these variants would agglutinate directly in heterologous sera.

On the basis of these findings it is recommended that *Proteus* X strains used in the Weil-Felix reaction be checked at least once a month to insure working with a nonmotile culture and at least once each quarter to exclude spontaneously occurring variants.

DIPHTHERIA: This Significance of the Schick Test in the Adult, Young, C. C., Bunney, W. E., Crooks, M., Cummings, G. D., and Forsbeck, F. C. Am. J. Pub. Health 24: 835, 1934.

A study of the relationship between the Schick reaction, antitoxin concentration, antigenic stimulation, and age in male adults, was made in two Michigan hospitals for the insane. The following observations were made from the data:

1. The results indicate that there is no antitoxin level in adults below which all Schick reactions are positive and above which all are negative.
2. Three-hundredths unit seems a satisfactory level to use as a dividing point. At any rate we are not able to suggest a better level.
3. "False negatives" and "false positives," basing these terms on the assumption that 0.03 unit or more means immunity and less than 0.03 unit susceptibility, occur in different proportions, before and after antigenic stimulation.

The Journal of Laboratory and Clinical Medicine

Vol. 21

August, 1936

No. 11

CLINICAL AND EXPERIMENTAL

RELIABILITY OF FERMENTATION TESTS IN IDENTIFICATION OF THE MONILIAS*

R. W. HOPKINS, PH.D., and H. CROSS HUSSEY, M.S., M.D., CHICAGO, ILL.

THE constancy in fermentation of sugars by the monilias is still controversial. One may find in the literature complete rejection of sugar fermentations or complete acceptance of retained ability to ferment certain sugars over a period of years.

Castellani¹ earlier proposed that the monilias be divided into species on the basis of their fermentative reactions, but later came to regard such reactions as by no means constant.² He stressed the importance of this determination as soon as possible after isolation. Ashford,³ Garrod and Stone,⁴ and Benham⁵ considered this method as not reliable for the classification of these organisms. Wachowiak and coworkers⁶ were unable to obtain consistent fermentation of sugars in simultaneous tests or in tests repeated over a period of years. Lamb and Lamb⁷ have expressed dissatisfaction with the usual method of determining sugars fermented by monilia and proposed chemical tests for sugar concerned as conclusive evidence of fermentation.

On the other hand, Thiermer⁸ and others have stated that the monilias may be classified by their few secretion reactions and that their ability to ferment given sugars is retained over a considerable period of time. Stevill and coworkers⁹ presented evidence that the monilias which they have isolated, as well as those obtained from culture collections, may be separated into three readily distinguishable groups using the method and physiologic criteria.

*From the Division of Infectious Diseases, Department of Medicine, University of Chicago School of Medicine, Chicago, Ill.
Supported by a grant from the National Cancer Institute.

EXPERIMENTATION

This report contains our observations on and an explanation of the variability in the fermentation reactions in monilias isolated from cases of oral thrush and vulvovaginitis. It has been established positively by Hesselstine¹⁴ and Plass and coworkers^{15, 16} that these organisms are clinically significant. The methods of classification of these organisms are discussed in another paper.¹⁷

The fact that one or more replicate tubes of the same sugar broth occasionally failed to show the production of gas led to a consideration of at least one of the factors operative in the inconstant fermentation characters reported for the monilias by some workers. Near the beginning of the work with these organisms, fermentation tests were run in triplicate in sugar broths with bromocresol purple and bromthymol blue as indicators. Except for the greater sensitivity of the bromthymol blue to changes in acidity, the results were identical.

The sugars were used in 1 per cent concentration in a broth containing: peptone 1.0 per cent, beef extract 0.35 per cent, and sodium chloride 0.5 per cent. Bromocresol purple was the regular indicator. The milk was made up by resuspending 90 gm. of skimmed milk powder in a liter of water, adding bromocresol purple in a concentration of 0.005 per cent,¹⁸ tubing, and sterilizing by heating for thirty minutes in an Arnold sterilizer on three successive days. After cooling, calcium lactate solution was added as given by Stovall and Bubolz.¹¹

Incubation was at 37° C. and observations were made on the second, third, fourth, and seventh days.

In the tests in which bromocresol purple was compared as an indicator with bromthymol blue, one or more of the six tubes of each sugar broth occasionally failed to show production of gas. The cultures showing such variations are reported in Table I. Out of 20 strains tested, 10 failed to produce gas in one or more tubes with one or more different sugars. The failure to produce gas occurred most frequently in those sugars which were not readily fermented.

TABLE I
FAILURE OF GAS PRODUCTION BY MONILIA IN REPLICATE FERMENTATIONS

CULTURE NUMBER	SUGARS			
	LEVULOSE	MANNOSE	GALACTOSE	MALTOSE
	TUBES OUT OF 6 SHOWING FAILURE OF GAS PRODUCTION			
262	1	3	Not fermented	0
262A	1	3	5	0
263	2	2	Not fermented	0
266	0	2	Not fermented	0
268	0	0	4	1
270	0	0	4	0
271	0	0	2	0
274	0	2	5	0
275	0	1	Not fermented	0
276	0	5	Not fermented	0

Glucose, the most easily fermented of the sugars tested, gave no failures. Maltose, which is fermented almost as readily as glucose, failed to produce gas in only one tube out of 120. In decreasing order of fermentability are levulose, mannose, and galactose. Galactose is fermented poorly, and was the most inconstant in gas production.

Several strains which had been used in earlier work were run through the fermentation tests. The results differed from those determined by another observer. Two of the most variable strains, 104 and 155, were inoculated into a series of the sugar broths by three persons. The inoculum was from tubes of parallel transfers on Sabouraud's agar. Table II gives the results of these tests. It is apparent that quite different results may be obtained by three persons working with the same strain, using the same batch of sugar broths, incubating for the same length of time, and making observations at similar intervals. The personal equation in reading the results was eliminated by having the readings checked by one person (C). The differences obtained in this test were believed to be due to a variable amount of inoculum. Accordingly, several tests were made using a large and a small inoculum.

The cultures selected for the experiments with variable amounts of inoculum were, except 56924 and 6508—030, those which had shown inconstant fermentation reactions in previous tests. Strains 303 and 305 were identified as *Monilia albicans*, Strains 306 and 6508—030 as *Monilia candida*, strain 56924 is an endomyces,* and 155 is an unidentified monilia, while Strains 300 and 304 were like *Monilia albicans* in most respects and probably belong to that species. The inoculum used was from an approximately forty eight-hour growth on a Sabouraud's glucose agar slant. The small inoculum filled only a very small arc of the circle of a 2 mm loop, while the large inoculum nearly filled it. Table III gives the results of this test. Gas appeared later and in smaller amounts in those tubes with the small inoculum than in those with the large inoculum. No gas was formed in some cases when a small inoculum was used. In two instances gas was produced when a small but not when a large inoculum was used.

Essentially the same test was performed with the inoculum grown in glucose broth for forty eight hours. A 0.05 cc and a 0.5 cc portion of the broth suspension of the organisms was used for the small and the large inoculum, respectively. This source of inoculum appeared to be more uniform in that there was no greater change produced by the small than the large inoculum. As in the previous test gas appeared earlier and in greater amounts when a larger portion was used, and with some strains gas was formed by the large inoculum only. Controls were run with distilled water and peptone broth containing fermentation tubes, in order to check whether gas was formed by any unused sugar remaining in the inoculum. No gas was formed in these controls. Another thought was that the large inoculum might introduce a sufficient number of cells with stored food reserves that gas could be produced from this source. No gas was formed in the absence of fermentable sugars by the organism from either the glucose broth suspensions or Sabouraud's glucose agar slants.

The data presented in Tables II, III, and IV indicate that the size of inoculum is apparently a factor in the variability of the fermentation reactions given by the monilias. This variation is most likely to appear in the less readily fermented sugars, especially galactose. With a weakly fermentable sugar, the evolution of gas would be slow, and might not exceed the limit of solubility in the medium. When more cells are present (large inoculum), the saturation point may be exceeded with gas collecting in the fermentation tube.

*We are indebted for this culture to Dr. W. D. Stovall of the Wisconsin State Laboratory of Hygiene, Madison, Wisconsin.

The results of fermentation tests on 7 strains of monilia at the time of isolation and after carrying in stock culture for six to eight months are reported

TABLE II

DIFFERENCES IN FERMENTATION REACTIONS OF MONILIAS DETERMINED BY THREE PERSONS

CULTURE NUMBER	PERSON	DEXTROSE	LEVULOSE	MANNULOSE	GALACTULOSE	SUCULOSE	MALTOSE	LACTULOSE	MILK
104	A	AG*	AG	AG	A	O	AG	O	C
	B	AG	A slight	(AG) slight	A	O	AG	O	C
	C	AG	AG	AG	A very slight	O	AG	O	C
155	A	AG	AG	A	O	AG	O	O	O
	B	A slight	O	O	O	O	O	O	O
	C	A	A slight	O	O	O	O	O	C

*A, acid; C, coagulated; G, gas; O, no change.

TABLE III

FERMENTATION REACTIONS OBTAINED WITH SMALL AND LARGE INOCULA FROM AGAR SLANT CULTURES

AG in Dextrose

CULTURE NUMBER		LEVULOSE	MANNULOSE	GALACTULOSE	SUCULOSE	MALTOSE	LACTULOSE	MILK
155	Small	AG*	AG	A slight	AG	O	O	C
	Large	A	AG	A slight	AG	O	O	C
300	Small	AG	AG	A slight	O	AG	O	C
	Large	AG	AG	AG	A very slight	AG	O	C
303	Small	AG	A (G) slight	A slight	O	AG	O	C
	Large	AG	AG	A	O	AG	O	C
304	Small	AG	AG	AG	O	AG	O	C
	Large	AG	AG	A	O	AG	O	C
305	Small	AG	AG	A very slight	O	AG	O	C
	Large	AG	AG	AG	O	AG	O	C
306	Small	AG	AG	AG	AG	AG	O	O
	Large	AG	AG	AG	AG	AG	O	O
6508 0.30	Small	A	AG	A	AG	AG	O	Alk.
	Large	AG	AG	A	AG	AG	O	Alk.
56924	Small	AG	AG	AG	AG	O	AG	C
	Large	AG	AG	AG	AG	O	AG	C

*A, acid; C, coagulated; G, gas; O, no change; Alk., alkaline.

in Table V. Formation of acid in bromocresol purple broth is interpreted by the authors as indicative of production of CO₂. The monilias are not known to produce organic acids as end-products of fermentation. "Acid" then indicates formation of a smaller amount of CO₂, while "acid and gas" indicates the formation of a larger amount. It could not then be said that any change had

occurred in the fermentative characters or sugars fermented by the group of organisms included in this test. An effort was made from the beginning of the work to inoculate fermentation tubes with what appeared to the eye to be similar masses of yeast cells. In spite of this crude method tests were duplicated.

In view of the reputed variability of the monilias, it was of interest to compare the fermentation results published by several workers with the groups proposed by Stovall and Bubolz.⁹ Table VI gives this data. Since we have not studied *Monilia parapsilosis*, we have no comments to offer about this species. *Monilia candida*, which ferments sugars more vigorously than *Monilia albicans*, has given no variation in the hands of several workers. The latter has given variable results only on those sugars fermented very slowly, galactose and sucrose. In our own work, acid production has been obtained in sucrose with only a very few strains. Nevertheless, the results, given in Table VI, are surprisingly consistent in view of the many factors which were varied by the several workers. In spite of this agreement, it is not proposed to classify the monilias by means of biochemical criteria alone. However, we do believe that biochemical criteria, in conjunction with morphology, are valuable in the classification of these fungi.

TABLE IV
FERMENTATION REACTIONS OBTAINED WITH SMALL AND LARGE INOCULA FROM
GLUCOSE BROTH CULTURES
AG in Dextrose and Levulose

CULTURE NUMBER		MANNULOSE	GALACTOSE	SUCROSE	MALTOSF	LACTOSE	MILK
104	Small	AG*	A slight	O	AG	O	C
	Large	AG	A	O	AG	O	C
155	Small	AG	A slight	AG	O	O	C
	Large	AG	AG	AG	O	O	C
300	Small	A very slight	A slight	A very slight	AG	O	C
	Large	AG	A slight	AG	AG	O	C
303	Small	AG	A	O	AG	O	C
	Large	AG	AG	A very slight	AG	O	C
304	Small	AG	AG	A	AG	O	C
	Large	AG	AG	A very slight	AG	O	C
6508 030	Small	AG	AG	AG	AG	O	Alk
	Large	AG	AG	AG	AG	O	Alk
56924	Small	AG	AG	AG	O	AG	C
	Large	AG	AG	AG	O	AG	C

*A acid C coagulated G gas O not run Alk alkaline

DISCUSSION

It appears from Tables II, III and IV that other factors than the size of the inoculum may be responsible for variation in the fermentation tests on monilias. The data in Tables III and IV were obtained by "C" of Table II. Note the differences between the fermentation reactions of 155 in the three tables. With Strain 300, gas was formed from galactose in the tests given in Table III and not in those of Table IV. Sucrose gave gas in one test and not in

the other. Similar differences are seen in Strains 303 and 304. As yet we have not had the opportunity to investigate other factors which may be operative in giving variable fermentation reactions. A few possibilities are: (a) the age of the inoculum, (b) the interval between previous transfers of the culture, and (c) the composition of the medium in which the inoculum is grown. If these factors were evaluated in relation to fermentation studies on the monilias,

TABLE V

FERMENTATION REACTIONS OF MONILIAS FRESHLY ISOLATED AND AFTER CARRYING IN STOCK CULTURE

AG in Dextrose, Levulose, Mannose, Maltose. No change in Lactose

CULTURE	DATE TESTED	GALACTOSE	SUCROSE	MILK
244	7/25/34	A (G)* very slight	A	C
	3/14/35	A (G) slight	A (G) slight	C
259	7/16/34	A (G) very slight	A	C
	3/14/35	A (G) slight	A (G) slight	C
262	7/31/34	A	A slight	C
	3/14/35	A slight	A very slight	C
264	7/31/34	A	O	C
	3/27/35	A very slight	O	C
265	7/31/34	A	A slight	C
	3/14/35	A slight	A (G) slight	C
268	8/16/34	AG	A slight	C
	3/14/35	A (G) slight	A slight	C
269	8/25/34	AG	AG	O
	3/14/35	AG	AG	O
271	9/14/34	AG	O	C
	3/27/35	A very slight	O	C
272	9/10/34	AG	A slight	C
	3/14/35	A (G) slight	A (G) very slight	C
274	9/17/34	A	O	C
	3/27/35	A slight	O	C
275	9/17/34	A slight	O	C
	3/27/35	A slight	O	C

*A, acid; C, coagulated; G, gas; O, no change.

greater unanimity might be promoted. Only a few cultures show variability; the remaining strains met in our work exhibit remarkably similar reactions representing only a few types of organisms.

In a recent paper Lamb and Lamb⁷ proposed that tests for disappearance of sugars be made in addition to the usual observation for production of acid and gas. These authors were dissatisfied with the use of bromeresol purple broth containing Durham tubes because of the reversion from an acid to a more alkaline reaction, occurring at different times in the different sugar broths. This reversion to a more alkaline reaction does not constitute a criticism of the method used. We have made tests which showed that this phenomenon is produced by a loss of CO₂ from the medium near the end of or after the completion

TABLE VI
MONILIAS GROUPED ACCORDING TO STOVALL AND BUBOLZ. FERMENTATION REACTIONS BY VARIOUS WORKERS

INDICATOR		DEXTROSE	LEVULOSE	MANNOSE	GALACTOSE	SUCROSE	MALTOSE
<i>Monilia parapsilosis</i> :							
Stovall and Bubolz	Bromthymol blue	AG*	AG	AG	A	A	A
Benham	Andrade	AG	AG	A	A	A	Not run
Lamb and Lamb	Andrade	AG	AG	Not run	A	A	O
<i>Monilia albicans</i> :							
Stovall and Bubolz	Bromthymol blue	AG	AG	AG	AG	A	AG
Benham	Andrade	AG	AG	AG	A irreg.	A irreg.	AG
Fineman	Not given	AG	AG	Not run	A	A	AG
Shrewsbury	pH determined	AG	AG	Not run	AG	A	AG
Lamb and Lamb	Andrade	AG	AG	Not run	A	A	AG
Lamb and Lamb (psilosis)	Andrade	AG	AG	Not run	AG	A	AG
Present authors	Bromeresol purple	AG	AG	AG	A or AG	A rarely	AG
<i>Monilia candida</i> :							
Stovall and Bubolz	Bromthymol blue	AG	AG	AG	AG	AG	AG
Benham	Andrade	AG	AG	AG	AG	AG	AG
Shrewsbury	pH determined	AG	AG	Not run	AG	AG	AG
Lamb and Lamb	Andrade	AG	AG	Not run	AG	AG	AG
Present authors	Bromeresol purple	AG	AG	AG	AG	AG	AG

*A, acid; G, gas; O, no change.

of fermentation. This reversion would appear at different times, depending on the rate at which the individual sugars were fermented.

Lamb and Lamb in comparing the disappearance of sugars with the fermentation readings of "acid and gas" demonstrated that sucrose and galactose disappeared from the medium, even though acid was the only indication of fermentation. With this method the less readily fermented sugars would require a longer incubation. In our opinion, the fermentation tests are more practical when a relatively short period is required for their completion. Employing the Durham tube method, and an indicator, as bromeresol purple, these tests can be completed in a week.

The reliability of the fermentation reactions of the monilias has been investigated and at least one cause of variability indicated. If the size of the inoculum is relatively constant, the difficulties encountered in obtaining consistent fermentation reactions may be lessened considerably. This point has been emphasized for the yeasts by Stelling-Dekker²⁰ and others. The difference between production of acid or acid and gas is only a difference of degree. However, under controlled conditions this difference can be of diagnostic value with but few exceptions. Thus the importance of a standard procedure for such work is further emphasized.

CONCLUSIONS

1. It is proposed that production of acid, or acid and gas, by monilias in sugar broths represents only a difference in degree and that both should be recognized as fermentation.

2. The rates of fermentations of most monilias are sufficiently different to permit separation into groups.

3. In some instances a large inoculum was found to produce fermentation when a small inoculum failed to do so.

4. Eleven strains of monilia had the same fermentation characteristics six to eight months after isolation.

5. A comparison of the fermentation data of several workers for the three types of monilia (Stovall and Bubolz) revealed considerable unanimity.

6. The occasional strains, which give variable fermentations, make morphologic study a desirable supplement.

REFERENCES

1. Castellani, A.: *Fungi and Fungous Diseases*, American Medical Association, 1928.
2. Ashford, B. K.: *The Etiology of Sprue*, *Am. J. M. Sc.* 154: 157, 1917.
3. Garrod, L. P., and Stone, K.: *The Classification of Monilias by Serological Methods*, *J. Path. & Bact.* 34: 429, 1931.
4. Benham, R. W.: *Certain Monilias Parasitic on Man. Their Identification by Morphology and by Agglutination*, *J. Infect. Dis.* 49: 183, 1931.
5. Benham, R. W.: *Monilias, Yeasts, and Cryptococci. Their Pathogenicity, Classification, and Identification*, *Am. J. Pub. Health* 22: 502, 1932.
6. Wachowiak, M., Marr, J., Hagebusch, O. E., Randall, W. A., and Fleischer, M. S.: *Differentiation of Various Strains of Monilia by Cultural Methods*, *J. Infect. Dis.* 54: 35, 1934.
7. Lamb, J. H., and Lamb, M. L.: *A Grouping of the Monilias by Fermentation and Precipitin Reactions*, *J. Infect. Dis.* 56: 8, 1935.
8. Fineman, B. C.: *A Study of the Thrush Parasite*, *J. Infect. Dis.* 28: 185, 1921.
9. Stovall, W. D., and Bubolz, A.: *Forty Strains of Yeast-Like Fungi Isolated From Sputum*, *J. Infect. Dis.* 45: 463, 1929.

- 10 Stovall, W D, and Bubolz, A Identification of Certain Funguses Pathogenic for Man, Am J Pub Health 22 493, 1932
- 11 Stovall, W D, and Bubolz, A Cultural and Biochemical Characteristics of Monilia Isolated From Human Sources, J Infect Dis 50 73, 1932
- 12 Stovall, W D, and Bubolz, A A Yeast Like Fungi, J LAB & CLIN MED 18 890, 1933
- 13 Stovall, W D, and Pessin, S B Classification and Pathogenicity of Certain Monilias, Am J Clin Path 3 347, 1933
- 14 Hesselstine, H C Diabetic or Mycotic Vulvovaginitis, J A M A 100 177, 1933
- 15 Plass, E D, Hesselstine, H C, and Borts, I H Monilia Vulvovaginitis, Am J Obst & Gynec 21 320, 1931
- 16 Hesselstine, H C, Borts, I H, and Plass, I D Pathogenicity of the Monilia (Castellani), Vaginitis and Oral Thrush, Am J Obst & Gynec 27 112, 1934
- 17 Hopkins, E W, and Hesselstine, H C Cultural and Morphological Studies of Cryptococci and Monilias Isolated From Vulvovaginitis and Oral Thrush, J LAB & CLIN MED 21 1113, 1936
- 18 Fred, E B, and Waksman, S A Laboratory Manual of General Microbiology, New York, 1928, p 39
- 19 Shrewsbury, J F D The Genus Monilia, J Path & Bact 38 313, 1934
- 20 Stelling Dekker, N M Die sporogeenen Hefen, Verh K Akad Wetensch Amsterdam Afd Natuurk 2 Sect Deel 17 No 1, pp 547

CULTURAL AND MORPHOLOGIC STUDIES OF CRYPTOCOCCI AND MONILIAS ISOLATED FROM VULVOVAGINITIS AND ORAL THRUSH*

E W HOPKINS, PH D, AND H CLOSE HESSELTINE, MS, M D, CHICAGO, ILL

A GROUP of yeastlike fungi, the monilias and cryptococci, which have been isolated from lesions in various parts of the human body, have received lately increased attention. The interest aroused has led to the proposal of a number of methods for identification and classification.

For a number of years, the system of Castellani,¹ which separated the monilias into species on the basis of the sugar fermentations, was accepted as the only means of identification. Timeman² reported that the fermentation reactions of the organisms which she studied were sufficiently constant to be used in identification. The large number of species which resulted from the use of Castellani's system and the difficulty in obtaining similar results on repetition led to numerous criticisms of this method. Later, Castellani¹ came to regard his fermentation method as being of little value unless applied to cultures of recent isolation. Nevertheless, he maintained that certain of the monilias did not change in their action on sugars.

Stovall and Bubolz^{3, 4} and Stovall and Pessin⁵ reopened the question of the use of a biochemical classification of the monilias. A number of cultures which they isolated from various lesions, as well as those obtained from stock collections, were found to be divisible into three groups. Malt agar and milk modified by the addition of calcium lactate were found to be important diagnostic aids in addition to the sugars commonly used. These strains were found to give the same reactions even after prolonged cultivation. No morphologic differences were observed which would allow the separation into species.

*From the Department of Obstetrics and Gynecology The University of Chicago.
Received for publication October 8 1935.
Supported by a grant from Standard Brands Inc.

The study of morphology of the monilias has been recommended by a few workers as a means of separating species. Ashford⁸ stressed the importance of these observations in addition to the fermentation tests. However, it is difficult to obtain exact morphologic descriptions from his paper. Benham^{9, 10} has made recently a distinct contribution in describing the morphology of four common monilia species. The type of growth and giant colonies were also found to possess differential characteristics. Fermentation of sugars was studied but doubt was expressed that sufficient differences were found to be of value, while serologic differences were believed to be the most definite and reliable. Benham and Hopkins¹¹ have applied the methods of Benham to monilias isolated from the fingers, toes, finger and toe nails, the tongue, and feces of 100 persons, and of the monilia cultures obtained, only 6 were not classifiable into four groups.

Langeron and Talice¹² have recently proposed a morphologic classification of yeastlike fungi. As yet, no papers have been found in which the key of Langeron and Talice has been applied to the monilias.

Serologic classification of the monilias has been advocated by several workers. Benham,⁹ and Benham and Hopkins¹¹ have reported success in distinguishing *Monilia parapsilosis*, *Monilia albicans*, *Monilia krusei*, and doubtful success with *Monilia candida* by means of agglutination and agglutinin absorption methods. Garrod and Stone,¹³ using complement fixation and precipitin tests, found most of the monilias in their collection to be identical with *Monilia albicans*. Almon and Stovall¹⁴ have reached the conclusion that the monilias cannot be separated by direct agglutination, but that by appropriate agglutinin absorption *Monilia parapsilosis* could be separated from *Monilia albicans* and *Monilia candida*. *Monilia albicans* and *Monilia candida* were not separable, as no specificity was exhibited toward these strains even by absorbed serums. Lamb and Lamb,¹⁵ reporting on precipitin reactions in the monilias, found 3 serological groups: (a) *Monilia albicans* and *Monilia candida*, (b) *Monilia parapsilosis* and (c) *Monilia krusei*. These workers believed that acid and gas formation in broth and disappearance of sugars could be used for separating the organisms into the same groups.

No attempt is made to review or clarify the confusion existing in the nomenclature which has developed about the pathogenic yeastlike organisms, since Stovall and Bubolz⁷ and Benham⁹ have adequately reviewed this subject. Until a decision by a special committee is given or the relationship of the yeastlike organisms has been more completely worked out, the name monilia seems to us to be as good as any other.

The yeastlike organisms isolated from vulvovaginitis and oral thrush were run through the fermentation tests and allowed to grow on agar plates for observation of morphology. The groupings determined by these two means are considered here, and the results obtained by the two methods compared.

EXPERIMENTAL DATA

Material from the vulvovaginal infection or oral thrush patches was removed with a swab or sterile instrument and smeared over a Sabouraud's dextrose agar slant. From those slants showing growth of yeastlike cells after one

or more days' incubation at 37° C material was streaked on Sabouraud's agar plates, and well isolated colonies picked. The pure culture so obtained was kept in stock culture on Sabouraud's agar.

As soon as possible after isolation, the pure cultures were inoculated from a forty eight hour, Sabouraud's agar slant culture into the sugar broths, 25 per cent malt broth, milk with additional calcium lactate,⁵ dilution plates made with 25 per cent malt agar,⁵ streaked on carrot plugs, and streaked on Sabouraud's and corn meal agar.

The sugar broths contained 1 per cent of a sugar, 1 per cent peptone, 0.35 per cent beef extract, and 0.5 per cent sodium chloride. Bromocresol purple was the indicator used except when bromthymol blue was tried for comparison. The milk was made up by resuspending 90 gm of skimmed milk powder in a liter of water and adding bromocresol purple to a concentration of 0.005 per cent.¹⁶ The milk was sterilized by heating for thirty minutes in an Arnold sterilizer on three successive days.

Fermentation tubes were incubated at 37° C and observed on the second, third, fourth, and seventh days. As has been pointed out in the preceding paper,¹⁷ the production of acid as well as acid and gas by the monilias is interpreted to be due to the formation of CO₂ from dissimilation of sugar, and is called fermentation. An attempt was made to use comparable inocula for the fermentation tests, since the size of inoculum, as indicated previously¹⁷ is an important factor. The estimation of the amount of yeast mass on a loop is a crude measure of the number of cells, however, after a few weeks, little difficulty should be encountered in obtaining consistent fermentations with all but a few cultures.

The organisms were grouped first on ability to produce mycelium on Sabouraud's agar plates. Those organisms failing to produce mycelium were called cryptococcus, and those producing mycelium, monilia. Since none of the cultures formed ascospores on suitable media, no tricharomyces or endomyces were isolated from this group of patients.

RESULTS

The sugars fermented by the cryptococci are given in Table I. Acid and gas were formed in dextrose, levulose, and mannose by the 8 strains, and acid and gas from maltose by one strain. None of the cultures made any perceptible change in bromocresol purple milk.

The results obtained with the monilias are given in Table II. These fungi are divided into the fermentation groups proposed by Stovall and Bubolz,³ and the type reactions given by these authors put into the table for comparison with the strains studied. None of the Type 1 monilias were found. Of the Type 2 monilias in 17 of the 67 strains, mycelium was observed in malt broth, but none formed on Sabouraud's agar. This latter type differed slightly in their reactions in sugar broths from those reported by Stovall and Bubolz. All strains produced acid in galactose broth, but formation of gas was variable. Acid in sucrose broth was formed by only a few strains.

Bromeresol purple had been selected as the indicator, because of its lesser sensitivity to slight changes in acidity. In the tests made in parallel with bromeresol purple and bromthymol blue, acid was produced in all cases in sucrose broth with the latter as the indicator, while with the former five strains failed to do so. Since Stovall has made use of the latter indicator, our differences in the production of acid in sucrose broth can be readily explained. However, as Lamb and Lamb have found that sucrose disappeared from broth inoculated with *Monilia albicans* it appears to be advisable for the determination of sugars attacked but not fermented to use an indicator changing at a higher pH than does bromeresol purple.

The coagulation of milk modified by the addition of calcium lactate as recommended by Stovall and Bubolz has proved to be a thoroughly reliable test. Strains of Type 2 monilia are occasionally encountered which do not coagulate this milk in three days, but will do so before the seven-day incubation period is completed.

TABLE I
FERMENTATION GROUPS OF THE CRYPTOCOCCI FOUND IN VULVOVAGINITIS

	NUMBER OF STRAINS	DEX-TROSE	LEVULOSE	MAN-NOSE	GALACTOSE	SUCROSE	MALTOSE	LACTOSE	MILK
Group 1	7	AG*	AG	AG	0	0	0	0	0
Group 2	1	AG	AG	AG	0	0	AG	0	0

*A, acid; G, gas; O, no change.

TABLE II
FERMENTATION GROUPS OF THE MONILIAS FOUND IN VULVOVAGINITIS AND ORAL THRUSH IN COMPARISON WITH TYPES OF STOVALL AND BUBOLZ

	NUMBER OF STRAINS	DEX-TROSE	LEVULOSE	MAN-NOSE	GALACTOSE	SUCROSE	MALTOSE	LACTOSE	MILK
Stovall and Bubolz									
Type 1		AG*	AG	AG	A	A	A	0	0
Type 2		AG	AG	AG	AG	A	AG	0	C
Type 3		AG	AG	AG	AG	AG	AG	0	0
Our Strains									
Type 1	0								
Type 2	67	AG	AG	AG	A or AG	A rarely	AG	0	C
Type 3	2	AG	AG	AG	A slight or AG	AG	AG	0	0
Unclassified									
Type 1	2	AG	AG	AG	A or AG	AG	AG	0	0
Type 2	2	AGP	AGP	AGP	slight 0	slight 0	0	0	0

*A, acid; C, coagulated; G, gas; P, pellicle.

One of the Type 3 strains produced only slight acidity in galactose broth, while the other strain gave the typical reactions of the Stovall and Bubolz Type 3. These two strains were not producing any symptoms or evidence of pathologic condition. One strain was isolated from the vulva of an apparently normal nonpregnant woman and the other from a stool of a woman without intestinal disturbances. These strains were sufficiently different in both fermentation reactions and morphology to be readily separated from Group 2.

Of the two unclassified groups, the first differed from Stovall's Type 2 only in the production of a slight amount of gas in sucrose broth, but as will be indicated later, this belongs in this Type 2. The second unclassified group appears to be closely related to *Monilia krusei*. It gives the fermentation results reported for that organism by Stovall.

Another criterion used by Stovall for the separation of groups of the monilias is the production of mycelium in forty-eight hours on malt agar. This test was made on about fifty strains. Of the Type 2 monilias tested, in four strains a few colonies showed the presence of some mycelium, and in the remaining strains none was found. The two Type 3 monilias produced abundant mycelium. It was observed, however, that even in strains producing abundant mycelium the formation of mycelium may not be a constant characteristic. It is affected by acidity and by a heavy or light seeding of plates. While this criterion may have some value in the differentiation of monilias when correlated with other tests, our experience has indicated that it should not be emphasized strongly.

An attempt was made to find morphologic groups in the series of cultures examined. The descriptions of Benham for *Monilia albicans* and *Monilia candida* were used as the types for classification into these species. These groups including the description of the types are given. A number of cultures formed mycelium only in malt broth but were not examined for morphology, as all other cultures had been observed on agar. In a few strains, the mycelium developed too poorly on agar to make possible morphologic studies. Eight strains were described insufficiently to make identification possible.

Monilia albicans Type—Hyphae short to elongate. Branching sometimes frequent. Buds in whorls which later submerge the hyphae, giving the appearance of a string of beads (27 strains).

Subtype of M. albicans—Like *M. albicans* except that the first buds may elongate, branch, and bear a cluster of conidia at a distance from the hyphae. This form of growth gives the hyphae a ragged appearance. These branches may later embed the hyphae, or the usual balls of conidia may be formed in other parts of the mycelium (4 strains).

Monilia candida Type—Mycelium very abundant. Buds may at first form in whorls and then other buds appear later between the whorls or the buds may form at irregular intervals from the start. The first buds elongate and branch, giving branched clusters at a distance from the hyphae (2 strains).

Miscellaneous types unlike the above two

1 Mycelium rather short, little branched. Buds elongate to ovate, placed at irregular intervals along the hyphae. Ragged appearance to old hyphae (2 strains).

2 Dense mycelium with branches of fir tree appearance. Conidia borne on these branches. Dry wrinkled colony growth, pellicle on sugar broths. Probably *Monilia krusei* (2 strains).

3 Mycelium may be much branched, made up of elongated elements constricted at the segments, or may have buds at irregular intervals which elongate and branch (2 strains).

iron to an exclusive milk diet protect against nutritional anemia and produce an apparent well-being in animals of the first generation, as judged by hemoglobin formation, growth, and reproduction. Underhill, Orten, Mugrage, and Lewis⁴ have observed no abnormalities in the organs of rats fed milk, copper, and iron for periods of more than a year; Waddell⁵ reported sterility in males of the first generation receiving a milk, copper, and iron diet. The sterility was marked by a progressive testicular degeneration coupled with an absence of motile sperm in the seminal fluid. Ferric chloride was supposedly the causative agent. He found marked degeneration of the testes of male rats as early as ten weeks on a milk, copper, and iron diet. Glanzmann⁶ observed that rats fed for a year on an exclusive milk diet grew well at first, but later growth slackened and became stationary. Atrophy of testes and mammary glands, coupled with a failure of reproduction and lactation, were noted. Cardiac hypertrophy also resulted. Reproduction and lactation were restored by manganese. Keil, Keil, and Nelson⁷ found that the animals on a milk, copper, and iron ration grow more slowly than those on a good stock diet. Reproduction occurred in animals of the first generation, but the second generation did not reproduce. This experience with second-generation rats, together with the findings of Waddell, led us to investigate both males and females of the first and second generations, in order to observe whether or not the apparent sterility on this diet could be due to a degeneration of sex organs. Metabolism studies on females receiving milk, copper, and iron were performed in an effort to bring out any indications of an organic failure when milk, copper, and iron constituted the diet.

EXPERIMENTAL

The results reported in this paper were obtained on rats. All animals were fed whole milk obtained from Holstein cows in the dairy herd of Iowa State College. The cows were fed a good dairy ration designed for maximum milk production and were milked by hand directly into glass jugs. This procedure offered a uniform milk supply and eliminated contamination by foreign matter. Purified copper as $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ and iron as $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ were added to the fresh milk each morning; the salts were made in the laboratory from the purified elements. Spectrographic examinations of the metals were made, in order to insure a high degree of purity. Hemoglobin was determined by the Newcomer method. The animals were bled by the tail.

The males in Table I were killed and the trimmed testes were weighed and placed in Bouin's fixing solution prior to histologic sectioning; the epididymis was cut and semen squeezed into a drop of Ringer's solution on a microscope slide which was immediately examined microscopically for motile sperm. Those animals on the longer experiments were first subjected to mating tests with unbred females from the stock colony. Many females of the second generation on a milk, copper, and iron diet were tested for permanent sterility at the end of an experiment by placing them with mature males reared on a stock diet. Other females were likewise tested but with this difference; all of the rats were fed stock ration during the mating test. The animals described in Table II were taken directly from the milk, copper, and iron diet, killed, and their ovaries

trimmed and weighed. All ovaries were fixed in Bouin's solution for histologic examination. The usual histologic technique for paraffin embedding was followed in the treatment of testes and ovaries. Sections 10 μ in thickness were stained with Delafield's hematoxylin and examined microscopically, some of the sections were counterstained with eosin, in an attempt to secure better differentiation.

TABLE I
WEIGHTS OF TESTES OF RATS ON DIFFERENT DIETS

FIRST GENERATION					SECOND GENERATION				
WK ON RA TION	BODY WT	WEIGHT OF TESTES	DONALD SON VALUES	AMT CU AND FE FED DAILY	WK ON RA TION	BODY WT	WEIGHT OF TESTES	DONALD SON VALUES	AMT CU AND FE FED DAILY
30	264	2.48	2.52	0.50 mg Fe + 0.05 mg Cu	32	254	2.16	2.48	0.50 mg Fe + 0.05 mg Cu
30	190	2.43	2.11		28	203	1.96	2.20	
42	290	1.43	2.65		28	210	2.10	2.24	
42	260	2.97	2.50		28	279	2.56	2.58	
44	225	2.45	2.37		28	330	2.76	2.79	
44	323	1.53	2.78		40	205	2.12	2.20	
44	385	1.81	3.00		40	214	2.72	2.67	
40	262	2.49	2.52		36	251	2.56	2.46	
36	234	2.39	2.37		44	221	2.41	2.31	5.0 mg Fe + 0.50 mg Cu
36	175	1.35	2.00		44	245	2.34	2.44	
36	300	2.94	2.69		40	213	2.32	2.24	
40	165	2.40	1.94		40	231	2.46	2.35	
40	172	2.26	1.98		40	188	2.54	2.09	
48	208	2.12	2.23	1.0 mg Fe + 0.05 mg Cu	Ave	234	2.42	2.38	Stock diet
20	213	2.21	2.24		40	180	2.30	2.05	
20	161	2.25	1.89		40	277	2.45	2.58	
20	131	1.47	1.63		40	255	2.27	2.48	
14	311	2.31	2.24		40	198	1.86	2.15	
14	163	2.25	1.92		Ave	227	2.22	2.31	
14	135	1.48	1.67						
Ave	223	2.15	2.26						

TABLE II
WEIGHTS OF OVARIES OF RATS ON DIFFERENT DIETS

FIRST GENERATION					SECOND GENERATION				
WK ON RA TION	BODY WT	WEIGHT OF OVARIES	DONALD SON VALUES	AMT CU AND FE FED DAILY	WK ON RA TION	BODY WT	WEIGHT OF OVARIES	DONALD SON VALUES	AMT CU AND FE FED DAILY
30	169	0.038	0.0479	0.50 mg Fe + 0.05 mg Cu	36	175	0.036	0.0482	0.50 mg Fe + 0.05 mg Cu
30	168	0.050	0.0479		36	180	0.046	0.0484	
30	160	0.036	0.0474		36	164	0.032	0.0476	
30	155	0.028	0.0471		36	180	0.040	0.0484	
30	175	0.044	0.0482		36	178	0.030	0.0483	
30	171	0.043	0.0479		36	186	0.036	0.0485	
30	150	0.027	0.0468		36	194	0.046	0.0489	
30	179	0.040	0.0483		36	165	0.031	0.0477	
36	187	0.034	0.0487		30	184	0.044	0.0485	
36	182	0.036	0.0484		30	158	0.034	0.0473	
36	185	0.040	0.0485		30	182	0.050	0.0484	
Ave	171	0.0387	0.0479		30	170	0.044	0.0479	
28	170	0.044	0.0479	Stock diet	30	148	0.036	0.0466	
28	161	0.040	0.0474		30	154	0.035	0.0471	
28	175	0.042	0.0482		30	170	0.044	0.0479	
30	187	0.046	0.0487		30	162	0.042	0.0474	
Ave	173	0.043	0.0480		30	155	0.046	0.0471	
					Ave	171	0.0395	0.0479	

3. A nitrogen partition in the urine of three groups of female rats fed stock ration, milk, and milk plus copper and iron respectively, shows changes in the excretion of ammonia, creatine, and creatinine nitrogen.

REFERENCES

1. Hart, E. B., Steenbock, H., Waddell, J., and Elvehjem, C. A.: Iron in Nutrition. VII. Copper as a Supplement to Iron for Hemoglobin Building in the Rat, *J. Biol. Chem.* 77: 797, 1928.
2. Keil, H. L., and Nelson, V. E.: The Role of Copper in Hemoglobin Regeneration and in Reproduction, *J. Biol. Chem.* 93: 49, 1931.
3. Keil, H. L., and Nelson, V. E.: The Effect of Various Colloidal and Crystalloidal Compounds in Nutritional Anemia of the Rat, *J. LAB. & CLIN. MED.* 19: 1083, 1934.
4. Underhill, F. A., Orten, J. M., Mudge, E. R., and Lewis, R. C.: The Effect of the Prolonged Feeding of a Milk-Iron-Copper Diet to Rats, *J. Biol. Chem.* 99: 469, 1932-33.
5. Waddell, J.: Male Sterility on Milk Diets, *J. Nutr.* 4: 67, 1931.
6. Glanzmann, E.: Experimentelle Untersuchungen über die Nahrungsdefekte der Kuhmilch und verschiedener Milchpräparate, *Ztschr. f. Vitaminforsch.* 3: 2, 1934.
7. Keil, H. L., Keil, H. H., and Nelson, V. E.: The Effect of Addition of Minerals and Sucrose to Milk as Shown by Growth, Fertility and Lactation of the Rat, *Am. J. Physiol.* 108: 215, 1934.
8. Hawk and Bergem: *Practical Physiological Chemistry*, ed. 10, Philadelphia, 1931, P. Blakiston's Son & Co.
9. Donaldson, H. H.: *The Rat*, ed. 2, Philadelphia, 1924, Wistar Institute of Anatomy and Biology.

 THE VALUE OF THE BIOPSY

J. MARSHALL NEELY, M.D., LINCOLN, NEB.

HISTORICAL

THE word biopsy comes from the Greek words meaning life and vision. At the present time the term is confined to examination of tissue removed from the living body in contrast to necropsy, meaning the examination of tissue from the dead body. Muller in 1838 showed tumors to be composed of cells. Kivisch (1847), Schuh (1851), and Thiersch (1865) employed the microscope to some extent in surgery. Virchow in 1854 demonstrated that the biopsy was of value but later was led to believe that the study of gross pathology was much more reliable in surgical pathology. His influence was so great at that time that progress in the histologic study of tumors was greatly retarded. It was Ruge (1879), however, who first adopted the microscopic examination of all surgical material, and it is he who is usually referred to as the "Father of the biopsy." He urged the careful microscopic study of all uterine curettings and also bits of the cervix uteri in all doubtful cases. His ideas were opposed by the medical profession of Germany probably due to the powerful influence of Virchow, and it was not until 1889 that the German Surgical Congress finally accepted his teachings. Since that time the biopsy has gradually become almost universally accepted, though there are still those who object to its use or use it too infrequently.

*From the Lincoln General Hospital.

Received for publication, October 17, 1935.

OBJECTIONS

As pointed out by McGraw and Hartman,⁸ the objections to the biopsy may be roughly divided into two classes (1) That the incision into the malignant neoplasm stimulates local growth and (2) that the trauma incident to incision stimulates dissemination and increases the incidence of distant metastasis. The experimental work of Knox,²⁷ Tyzzer,²⁸ Lubarsch,²⁴ and Wood²⁶ has done much to discredit these objections. Lubarsch traumatized implanted areas of mouse sarcoma following which he could find no evidence of increased metastasis. Wood, incising into Flexner rat carcinomas found no increase in metastasis. On the other hand, Tyzzer and Knox demonstrated that massage of experimental mouse or rat tumors did cause a dissemination of tumor tissue into the lungs.

In spite of this experimental work there are some who believe the biopsy a dangerous procedure and, in certain instances which will be discussed more fully later, the biopsy is a procedure which should be done only after every other possible clinical and laboratory study has failed to make the diagnosis. Authorities differ widely as to the indications and contraindications of the biopsy. Ewing³⁰ regarded curettage as a dangerous procedure in cases of carcinoma of the fundus uteri. Bloodgood denied any danger associated with biopsies of the cervix or corpus uteri. Most authorities now regard diagnostic curettage and cervical biopsies as being indicated in any questionable case. Bloodgood¹⁴ in a long series of cases could find no evidence of increased metastasis, or decrease in five year cures in those breast tumors which had been biopsied.

TECHNICAL CONSIDERATIONS

Welch is given credit for having made the first frozen section in 1891, though Wilson, McCarty, and Bloodgood have been largely responsible for its development since that time. There is a wide variance of opinion as to the efficacy of the frozen section, even at the present time. Warthin¹⁵ as late as 1931 regarded the frozen section as a passing fad and believed paraffin sections much more reliable. At that time he described a twenty four hour technic, which is now being used in the Pathology Department at the University of Michigan. Others (McCarty and Biodeis) place just as much confidence in the rapid frozen section as they do in paraffin sections, and even go so far as to state that in some instances the preparations are more reliable. Thus, they say, is particularly true in case of unfixed tissue where they believe the cell detail is less distorted by artifacts. In any event, the technic used in the preparation of tissues for diagnosis should be chosen to best fit the training of the pathologist. If the frozen section is used it should in every instance be followed by a paraffin section.

Close cooperation between the surgeon and pathologist is essential for accurate tissue diagnosis. The pathologist must be able to orient the tissue which is sent to him for examination. The presence of the pathologist at the operating table at the time of the biopsy is the ideal way to accomplish this end. Too often the surgeon sends a very small bit of tissue to the laboratory when a more generous piece could as well be had. Thus, in many instances, makes diagnosis impossible. Rather than to feel it a duty to render an opinion in such a case, it is much safer for all concerned to insist on more material or decline to give an opinion.

SPECIAL REGIONS

No hard and fast rules can be laid down as regards indications and contraindications to the biopsy, but each case and each region under consideration should constitute a separate problem. Again various authorities with wide experience differ markedly in their methods of handling tumors of various organs. Each of the tissues from which neoplasms commonly arise will be briefly considered.

Perhaps the most common region from which biopsies are taken in a general surgical service is the female breast. In many instances, a surgeon of experience does not require a tissue diagnosis in cases of carcinoma of the breast, because the gross pathology is so characteristic. However, there are pathologic entities such as plasma cell mastitis, which so closely resemble acute carcinoma of the breast that a microscopic diagnosis is essential. Fat necrosis is also often misleading so far as the gross picture is concerned. If the tumor is to be examined histologically the entire mass should be removed in order that the pathologist may select the most questionable area for microscopic study. If the frozen section is used it should be done immediately and a report made to the surgeon. If the paraffin method is used, the wound may be sutured and the breast amputated if necessary at a later date. Bloodgood considers two weeks a safe interval between biopsy and radical surgery. It is no longer considered good surgery to remove a portion of a breast tumor for microscopic examination. The reason for this is not the effect of incision on the tumor but because such a small bit of tumor is not a true index of the character of the tumor as a whole.

Interpretation of histologic changes occurring in lymphoid tissue taxes the powers of the most experienced pathologist. The dividing line between inflammatory lesions and early changes seen in some of the lymphoblastoma group is extremely difficult to define. This tissue should always be fixed in Zenker-Formol solution as well as formalin and, unless the diagnosis is evident in the hematoxylin and eosin stain, one of the blood stains such as Dominici or Giemsa should be done. I have found histologic diagnosis on sections made by the frozen method to be extremely unreliable.

With the development of the transurethral resectoscope came a marked increase in the amount of prostatic tissue available for microscopic study. Clinically unsuspected carcinoma of the prostate is not infrequently discovered as a result of routine examination of this tissue. As pointed out by Munger and Angle,²² the percentage of carcinoma of the prostate has increased since the use of the transurethral resectoscope, their percentage being 13.5 of all cases.

The purpose of microscopic study of tissue removed from the stomach is usually to prove the presence or absence of malignancy. In questionable cases the use of the frozen section is very valuable here, as it is important for the surgeon to know whether or not malignancy exists before proceeding. The choice between gastric resection and simple excision or gastroenterostomy is often made as the result of the pathologist's report. In the face of a negative report, serial sections of a callous ulcer of the stomach may later show carcinoma microscopically in the paraffin sections.

Care should be taken in taking biopsies of the rectum. It is quite essential that the block be taken perpendicular to the mucosa. There are no contraindications to taking biopsies in this region.

Biopsy is the only means of absolute diagnosis of carcinoma of the esophagus. This should be done in each case in which there is a suspicion of malignancy, in view of the fact that the roentgen signs are far from being 100 per cent accurate. Esophageal ulcer and cardiospasm often produce x-ray shadows which are many times wrongly diagnosed carcinoma by competent roentgenologists.

Biopsies of tumors occurring in the main bronchi and trachea should be done whenever the x-ray film of the chest shows pathology located at a point where it is accessible to the bronchoscope. It is of value both from a diagnostic and prognostic standpoint. In those cases where the x-ray shows a questionable neoplasm near the pleura aspiration biopsy may be done.

Histologic examination of neoplasms of the skin is particularly important. Whether or not the entire lesion is excised for study depends somewhat on its character and location. If on the face, and appearing to be a basal cell carcinoma either a portion or all of it may be removed, preferably the entire tumor if it is small. If the lesion shows evidence of belonging to the melanoma group, it should be widely excised and the entire specimen examined histologically. While it is impractical to remove every nevus encountered, it is usually thought best to examine in this manner those exposed to much irritation. In cases of diffuse neurofibromatosis, or Von Recklinghausen's disease, one should remove tumor tissue with a great deal of hesitancy as surgery is followed by malignant transformation in a rather large number of instances.

Small tumors occurring in the mouth should always be removed and examined histologically as many of them show malignant change at an early stage. If the tumor is large, a portion of it should be removed for section.

Microscopic study of lesions occurring on the cervix uteri and in the endometrium is always indicated where there is any suspicion of malignancy. It was these tissues which were first studied by Ruge. In the case of biopsies of the cervix it is essential that the block be removed so as to represent a piece of tissue perpendicular to the mucosa. A small bit of tissue carelessly removed often shows many bizarre and misleading microscopic changes. There are many pitfalls in the microscopic diagnosis of early squamous cell carcinoma of the cervix, even when great care is taken by the surgeon in the removal of the block. Curettage is now one of our most commonly used diagnostic procedures. There is no evidence at the present time that diagnostic curettage is contraindicated in cases of carcinoma of the uterine fundus. It is the only known method to make an accurate diagnosis.

Bloodgood¹⁴ pointed out that biopsy in bone tumors should be done only after every other possible diagnostic means has been exhausted, such as x-ray, Wassermann, response to radiation, etc. Rapid plunging into the biopsy before careful clinical study of the case should be unreservedly condemned. The diagnosis of some bone tumors, such as Ewing tumor, multiple myeloma, and some cases of osteogenic sarcoma when based on histology alone is extremely difficult. A pathologic diagnosis based on the histology plus the objective and subjective

clinical findings is much more reliable. Response to radiation as a diagnostic test is very valuable and is indicated particularly where such radiosensitive bone neoplasms, as Ewing tumor or multiple myeloma, are suspected.

CLINICAL VALUE

Biopsy as a diagnostic aid in other than neoplastic diseases is of distinct use in selected cases. Special instruments such as that proposed by Randall¹ have been advocated in cases of endometrial biopsies in which instance it is unnecessary to give any anesthetic. Such a common diagnosis as hyperplastic endometrium, which has recently been shown to be the result of excessive estrogenic hormone, may thus be made with very little inconvenience to the patient.

The study of bone marrow microscopically has been used particularly during the past few years. As in the case of lymph nodes, the most valuable preparations are obtained if special technic is used, including Zenker fixation and staining with Giemsa's stain. Touch preparations such as advocated by Downey stained with double strength Giemsa make beautiful preparations and may, in certain instances be the sole means of making a diagnosis of such difficult entities as the so-called aleucemic leucemia and other blood dyscrasias. This is not a formidable procedure and can be done without causing the patient any great inconvenience. A button is removed from the sternum, touch preparations being made immediately from the undersurface. If there is sufficient tissue, paraffin sections can also be made from the bone marrow. This procedure is of value not only from the standpoint of leucemia but also in obscure cases of anemia.

Small bits of tissue removed from the liver for microscopic examination frequently bring to light the true nature of some obscure cases of intrahepatic pathology. Such an examination is of particular value in such cases as hemochromatosis, early cirrhosis, etc. Graham³¹ took biopsies of the liver in patients operated upon for gallbladder disease, in order to determine the histologic changes in cases of chronic cholecystitis.

The splenic puncture is a procedure which is of distinct value in selected cases where the underlying pathology is not readily detected in the clinical study. Such diseases or syndromes, as Gaucher's disease, Banti's disease and aleucemic leucemia, may be thus diagnosed where all other methods fail.

Biopsies of skeletal muscle have been used recently in the detection of arteriosclerosis in cases of hypertension and in suspected trichinosis.

Besides its diagnostic value, the biopsy may furnish other important information. The degree of differentiation of malignant tumors determined histologically is very valuable as regards prognosis. Certain histologic criteria may predict the radiosensitivity. Microscopic study of metastatic tumor may give a clue as to the point of origin. There is marked difference of opinion as regards the value of grading of tumors as proposed by Broders of the Mayo Clinic. Some believe that it has prognostic significance.

Minute, clinically unsuspected areas of malignancy will be discovered only if all surgical tissue be examined in the pathology laboratory. Such lesions as carcinoid appendix, gastrointestinal tuberculosis, and abdominal Hodgkin's disease may be discovered by the routine examination of the appendix. Microscopic findings in the tonsils, characteristic of the prodromal stage of measles,

have been described by Warthin and others. These would not have been discovered had it not been for routine tonsil examination. An occasional clinically unsuspected lymphosarcoma or carcinoma of the tonsil is also disclosed. Many other reasons can be named for routine tissue examination, which is gradually being accepted as a part of every standard hospital.

As pointed out by Zemansky and others the sectioning and histologic examination of pleural and ascitic fluids often reveals tumor cells in cases of occult carcinoma. The technique is simple and each fluid from a case in which there is suspicion of an existing tumor should be so studied.

That the popularity of the biopsy is gradually gaining ground is evidenced by the recent development of special instruments and methods of procuring tissue. Hoffman⁹ recently devised a punch for the removal of small bits of tissue from tumors for microscopic diagnosis. Others^{3, 4, 17, 20} have described their findings and the advantages of obtaining tissue by means of aspirations of suspected tumor material. This method is very simple and can be done in any office without causing the patient any discomfort. It is particularly applicable in cases of deep seated tumors. The technique consists of plunging a large needle into the suspected tumor mass, after having first made a nick in the skin, and applying suction as the needle is felt to enter the tumor. The contents of the needle are then blown into a test tube of 10 per cent formalin and the tissue run through as a regular section. This method has been used in breast tumors, bone tumors, suspicious prostatic neoplasms, and in some instances in lymph nodes. It has also been used in lung tumors, spleens, and deep seated muscle and fascia tumors.

It is important for the pathologist to have an accurate knowledge of the clinical data in each case from which tissue is taken for study. Histologic changes must be weighed in the balance, considering such factors as age, sex, length of illness, etc.

CONCLUSIONS

- 1 The biopsy is now recognized as a standard diagnostic procedure
- 2 Close cooperation between the surgeon and pathologist is essential to accurate diagnosis
- 3 Routine histologic examination of all surgical tissues is an essential requirement of every standard hospital
- 4 The biopsy should in every instance be preceded by a thorough clinical study
- 5 Recently developed methods of doing biopsies are reviewed

REFERENCES

- 1 Randall, I. M. Endometrial Biopsy, *Proc. Staff Meetings Mayo Clinic* 10: 143, 1935
- 2 Budd, S. W. *Internat. J. Med. & Surg.* 47: 490, 1934
- 3 Martin, H. E., and Ellis, E. B. Aspiration Biopsy, *Surg. Gynec. Obst.* 59: 578, 1934
- 4 Friedman, M. Clinical Value of Puncture Biopsies, *Radiology* 23: 429, 1934
- 5 Coisser, R. M. Consideration of Tissue Diagnosis, *M. Ann. Dist. of Columbia*
- 6 Guequierre, J. P., and Weidman, F. D. High Frequency Currents in Performing Biopsies, *J. A. M. A.* 103: 1693, 1934
- 7 Doege, P. F. Pros and Cons of the Biopsy, *Wisconsin M. J.* 33: 184, 1934
- 8 McGraw, A. B., and Hartman, T. W. Present Status of the Biopsy, *J. A. M. A.* 101: 1205, 1933
- 9 Hoffman, W. J. Punch Biopsy in Tumor Diagnosis, *Surg. Gynec. Obst.* 56: 829, 1933

10. Roussy, G., and Leroux, R.: Value of the Biopsy for Physician and Surgeon, *J. Med. frac.* 22: 88, 1933.
11. Shafiroff, B. G. P.: Biopsy Question, *Am. Med.* 37: 605, 1931.
12. Hellwig, C. A.: Biopsy in Tumors, *Arch. Path.* 13: 607, 1932.
13. Donaldson, F.: The Practical Application of the Microscope to the Diagnosis of Cancer, *Am. J. M. Sc.* 25: 43, 1853.
14. Bloodgood, J. C.: Biopsy in the Treatment of Malignancy, *J. LAB. & CLIN. MED.* 16: 692, 1931.
15. Warthin, A. S.: The Clinical Laboratory as an Aid to Surgery, *J. LAB. & CLIN. MED.* 16: 743, 1931.
16. Zemansky, A. P. J.: Examination of Fluids for Tumor Cells, *Am. J. Med. Sc.* 175: 489, 1928.
17. Martin, H. E., and Ellis, E. B.: Biopsy by Needle Puncture and Aspiration, *Ann. Surg.* 92: 169, 1930.
18. Stewart, F. W.: Diagnosis of Tumors by Aspiration, *Am. J. Path.* 9: 801, 1933.
19. Ferguson, R. S.: Prostatic Neoplasms, Their Diagnosis by Needle Puncture and Aspiration, *Am. J. Surg.* 9: 507, 1930.
20. Coley, B. L., Sharp, G. S., and Ellis, E. B.: Diagnosis of Bone Tumors by Aspiration, *Am. J. Surg.* 13: 215, 1931.
21. Bloodgood, J. C.: Danger of Incomplete Removal of Small and Apparently Innocent Lesions and the Problems of the Biopsy, *Surg. Gynec. Obst.* 44: 413, 1927.
22. MacCarty, W. C.: Indications and Rules for Biopsy, *Proc. Staff Meetings Mayo Clinic* 4: 61, 1929.
23. Tyzzer, E. E.: Factors in the Production of Metastasis, *J. M. Research* 28: 309, 1913.
24. Lubarsch, O.: The Significance of Trauma in the Origin and Growth of Malignant Tumors, *Med. Klin.* 8: 1651, 1912.
25. Knox, L. C.: Trauma and Tumors, *Arch. Path.* 7: 274, 1929.
26. Wood, F. C.: Diagnostic Incision of Tumors, *J. A. M. A.* 73: 764, 1919.
27. Knox, L. C.: Relationship of Massage to Metastasis in Malignant Tumors, *Ann. Surg.* 75: 129, 1922.
28. Terry, B. T.: A New and Rapid Method of Examining Tissue Microscopically for Malignancy, *J. LAB. & CLIN. MED.* 13: 550, 1928.
29. Didgeon, L. S., and Patrick, C. V.: A New Method for Microscopic Diagnosis of Tumor, *Brit. J. Surg.* 15: 250, 1927.
30. Ewing, James: The Incision of Tumor for Diagnosis, *New York M. J.* 102: 10, 1915.
31. Graham, E. A.: Hepatitis. A Constant Accompaniment of Cholecystitis, *Surg. Gynec. Obst.* 26: 521, 1918.
32. Munger, A. D., and Angle, E. E.: Transurethral Resection of the Prostate With Special Reference to the Follow-Up, *Nebraska State M. J.* 20: 326, 1935.

DOES CALCIUM NEUTRALIZE THYROXINE?^{*}

C. ALEXANDER HELLEWIG, M.D., WICHITA, KANS.

WHILE iodine deficiency is regarded by most goiter students as the essential cause of endemic goiter, the contention that a positive factor is necessary for the development of goiter is more and more recognized.

Recent experimental work of Tanabe,¹ Hellewig,² Thompson,³ and Remington and von Kolnitz⁴ have established the fact that an excessive intake of calcium is a powerful goitrogenic agent. By giving a diet rich in calcium and, at the same time, containing different amounts of iodine, I⁵ was able to produce in white rats parenchymatous and colloid goiters.

The part that calcium plays in goiter development is not understood. There is the possibility, as Marine⁶ believes, that it acts by neutralizing thyroxine, that is, by creating a relative insufficiency of iodine. Zondek and Reiter⁷ observed in tadpoles which were kept in a 1:15 million dilution of thyroxine a precipitate metamorphosis as first described by Gudernatsch.⁸ By increasing the calcium content of the water, Zondek and Reiter saw the thyroxine effect getting weaker and weaker, until finally, when the 400 c.c. of water contained 0.4 gm. of calcium chloride and 0.02 mg. of thyroxine, the tadpoles developed more slowly and grew faster than the controls. Calcium in high dose, in their experiments, reversed the thyroxine effect and brought about changes as observed after feeding thymus substance.

These results, if confirmed, would indeed offer a simple biochemical explanation of the goitrogenic effect of calcium. Goiters caused by an excessive intake of calcium could then be regarded as work hypertrophy of the thyroid in response to a relative iodine deficiency.

Since the photographs which accompany Zondek and Reiter's article do not show a definite influence of calcium on the thyroxine effect, a repetition of these experiments seemed indicated. Three hundred larvae of a common toad were used in my study on which this paper is based. All tadpoles were obtained from a single pool and were equal in size and stage of development. They had no extremities and the tail was large, fanlike. One hundred tadpoles served as controls and were placed in 3,000 c.c. of city water. The second group of 100 were kept in 3,000 c.c. of water containing 0.2 mg. of thyroxine, and the third lot was placed in the same amount of water with thyroxine, but with an addition of 4 gm. of calcium chloride. The food was the same for all three groups and consisted of ground meat and wheat gluten.

Representative specimens of each group, at the end of the experiment, are shown in Fig. 1. While after five days the controls still had all the charac-

^{*}From the Department of Pathology of St. Francis Hospital.
Received for publication, September 28, 1935.

teristics of tadpoles, the metamorphosis of the animals in the second and third groups was almost completed. It is evident that the thyroxine effect was not altered in the least by the addition of calcium. Also the decrease in size was

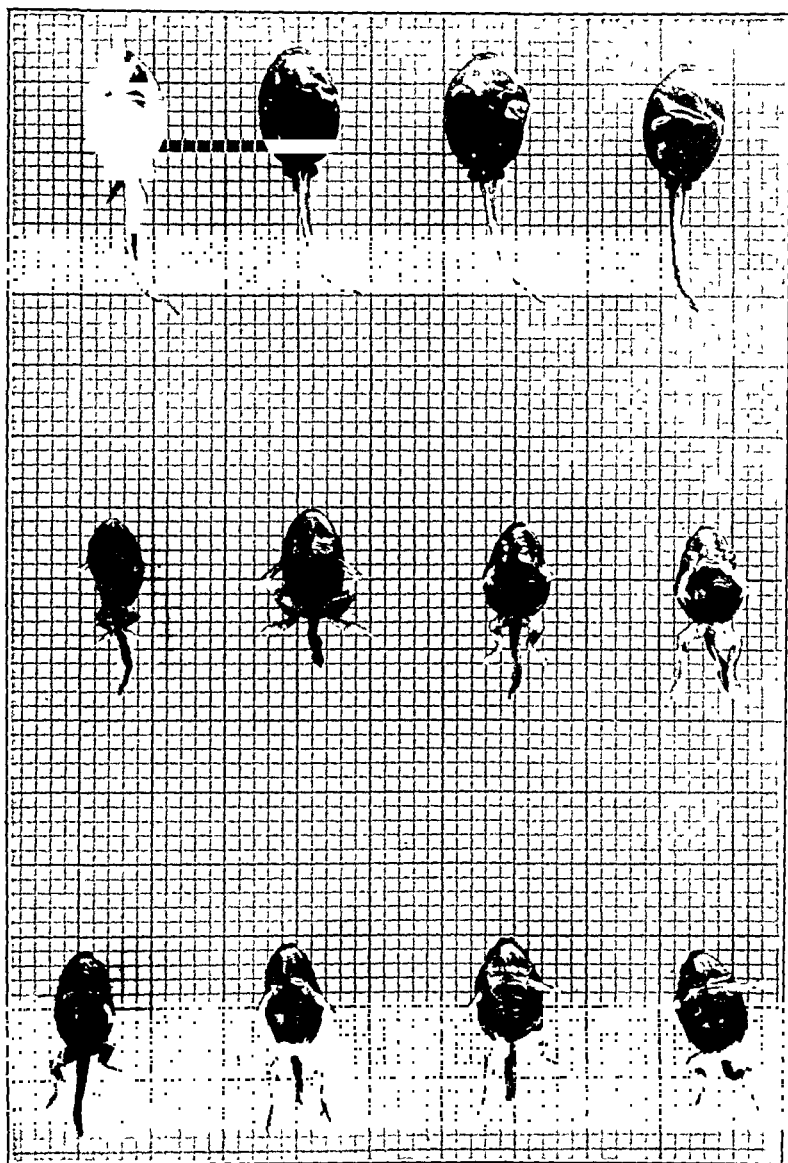


Fig. 1.—Representative specimens of the three experimental groups, killed at the end of the feeding period. First row: Controls. Second row: Thyroxine group. Third row: Thyroxine-calcium group. No difference is noticed between the second and third group.

the same whether calcium was added to the thyroxine or not. The average weight of the controls, at the end of the feeding period, was 800 mg., that of the thyroxine group 343 mg., and that of the thyroxine calcium group 335 mg. Added calcium did not lessen the toxicity of thyroxine. While of the con-

ADMINISTRATION OF EPINEPHRINE BY INHALATION*

JAMES B. GRAESER, M.D., AND ALBERT H. ROWE, M.D., OAKLAND, CALIF.

OF ALL drugs which have been used for the relief of asthmatic paroxysms, epinephrine remains supreme. Its use in this field has been principally confined to administration by hypodermic injection. Recent investigations by the authors,¹ however, have demonstrated the feasibility of obtaining comparable, and in many instances superior, results by inhalation. Success with the latter method is dependent on two factors: first, the use of a stronger epinephrine solution, preferably a 1:100 dilution, and second, an atomizer capable of delivering a fine vapor-like spray.

For nebulization of the 1:100 solution, the majority of the available atomizers were found to be unsuitable, either because of the presence of metal parts

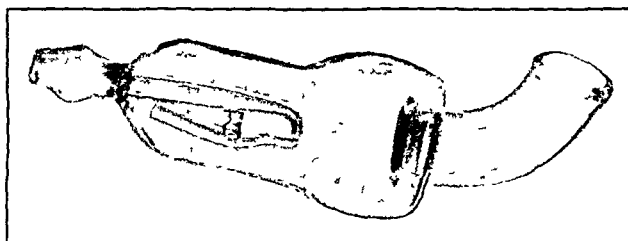


Fig. 1.—Hand atomizer for administration of 1:100 epinephrine solution by inhalation.

which oxidize the epinephrine, or because of certain features of design, or the unevenness of the delivered spray. The instrument, Fig. 1, finally designed was so constructed that satisfactory nebulization could be obtained with but eight or ten drops of solution, a feature desirable in that it conserves the drug by minimizing the amount of the solution exposed to oxidation. Furthermore an inverted moat was incorporated into the instrument to prevent spilling during use, for in the earlier trials several patients accidentally swallowed some of the solution and suffered severe abdominal pain as a result. An ordinary rubber bulb furnishes the air pressure for nebulization.

The method of using the atomizer is quite simple but requires, nevertheless, a modicum of coordination which occasional patients find difficult to acquire. The nozzle of the atomizer is placed just within the open mouth, and the patient inhales deeply while creating a spray. The amount of inhalation necessary for relief varies for each patient and depends not only on the severity of the symptoms, but also on the manner in which the atomizer is manipulated. There is a wide margin of tolerance before any unusual reactions occur. However, until familiar with the procedure, the patient is advised to proceed slowly, allowing one to two minutes to elapse between every five or six deep inhalations.

*Received for publication, October 1, 1935.

For use with children unable to cooperate in the use of the hand atomizer, a special apparatus was devised. This includes first of all, a mask designed to

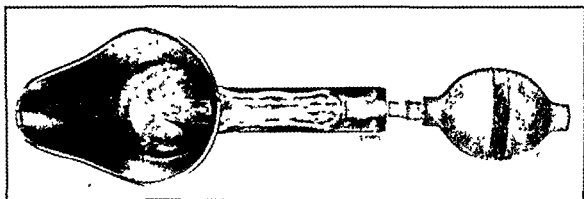


Fig. 2—Atomizer and metal face mask, for inhalation of adrenalin by infants and children

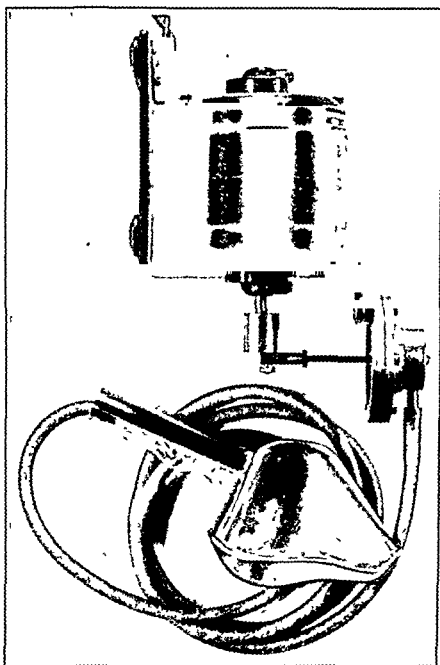


Fig. 1—The portable air compressor, mask and atomizer for prolonged administration of adrenalin by inhalation

fit the face, covering the nose and mouth. An atomizer of somewhat different external design fits into the handle with the nozzle projecting into the mask and directed toward its closed end. Air pressure may then be furnished either by

the rubber bulb, Fig. 2, or by a small motor-driven pump, Fig. 3. With either of these instruments, a large volume of vapor may be maintained in the mask while being held over the face of the patient. Its advantages with small children are obvious, and in actual practice, it is an easy matter to administer satisfactorily the solution to small noncooperative patients. The motor apparatus is extremely useful in severe attacks, when medication is needed at frequent intervals. It also fulfills a need in adults when nursing care is not available, and the patient is so weakened by asthmatic distress that the exertion of using the hand bulb is exhausting. The time of exposure with the mask apparatus varies as with the hand instrument, depending principally, however, on the severity of symptoms. Fifteen seconds is the minimum exposure but as much as three minutes have been used in a single exposure, without unusual symptoms of toxicity. In general, less than a minute usually suffices for a single treatment.

The results obtained by the inhalation procedure have been almost uniformly successful. Failure to obtain initial relief is usually due to improper manipulation of the atomizer or to poorly constructed instruments. Three hundred and fifty individuals of all ages, from eleven months to eighty years, have so far been instructed in the use of this inhalation technic. Attacks of all grades of severity have been treated. Severe paroxysms require more prolonged exposure and times intermittently for a period of fifteen to thirty minutes before relief is obtained. In these instances, however, the decreased tidal air prevents inhalation of an amount of the drug sufficient to give relief in the usual time. The ideal routine in such cases is the administration of hypodermic epinephrine to obtain initial relaxation. This is followed by the use of the inhalation procedure on experiencing the least suggestion of a return of symptoms.

With the average patient, the physiologic effect occurs more rapidly after inhalation of the 1:100 solution than with hypodermic injection; rarely is it any slower. Inhalation is infrequently accompanied by any of the disagreeable side effects of subcutaneously administered epinephrine. So satisfactory have been the results obtained, that the authors feel the inhalation procedure may well supplant in many instances, at least, the use of epinephrine subcutaneously. Furthermore its superiority to the numerous proprietary remedies administered by inhalation should allow the medical profession to combat more adequately the widespread use of these nostrums.

REFERENCE

1. Graesser, J. B., and Rowe, A. H.: Inhalation of Adrenalin for the Relief of Asthma, *J. Allergy* 6: 415, 1935.

242 MOSS AVENUE

PRODUCTION OF THE BASOPHILIA OF THE GRANULES OF THE POLYMORPHONUCLEAR NEUTROPHILIC LEUCOCYTES IN EXPERIMENTAL INFECTION OF THE RAT*

SISTER M. FRANCIS XAVIER O'REILLY, R.S.M. M.S., ANN ARBOR, MICH.

IN CERTAIN infections in man, the granules of the polymorphonuclear neutrophilic leucocytes have a greater affinity for methylene blue than have normal neutrophils. This basophilia of the granules appears to be a definite indication of infection, but it may be seen in other blood conditions and also follow x-ray therapy. Certain problems relating to the onset of the change of the staining character of the granules, the duration, relation to the types of infecting organism, and the appearance of the phenomenon in the blood of organisms other than man are of interest.

Cesaris Demel¹ was the first to describe the difference in staining quality and increased granulation of the polymorphonuclear neutrophils.

Mommsen,² Gloor,³ and others have demonstrated the presence of these deeply staining "toxic" granules in lobar pneumonia. Mommsen used a buffered solution of pH 5.4 with Giemsa's stain to demonstrate the presence of these granules. He termed them "pathologic" or "toxic" granules.

Matis⁴ believed Mommsen's granules to be identical with those described by Naegeli as "toxic" or "toxic infectious" granules.

Rosenthal and Sutro⁵ noted two kinds of granules: small granules and large granules. They found the small ones in mild infection and during convalescence, and the larger "toxic" granules in severe infection. They believe these pathologic cytoplasmic alterations ("toxic granules") are a valuable aid in the diagnosis and prognosis of pneumonia.

From a study of cytoplasmic changes in the peripheral neutrophils, Meranze, Mendell, and Meranze⁶ found "toxic" changes in pneumonia, peritonitis, bacteremias, and septicemias, generalized types of infection. In localized infections and noninfectious diseases, the neutrophilic leucocytes did not manifest these changes.

Sutro⁷ found the presence of "toxic" granules a constant factor in generalized infection. Spaeth⁸ believed that the cytoplasmic changes depend on the virulence of the infecting organism. Fitzhugh⁹ used the basophilia of the cytoplasmic granulation of the neutrophilic series of leucocytes as a criterion of the age of the neutrophils.

The present study was undertaken to produce these cytoplasmic changes in the polymorphonuclear neutrophilic leucocytes of the peripheral blood of the rat. The points considered in the experiment were: When do the changes appear? Do they appear in the older (multilobed) forms or in the younger

*From the Department of Zoology and the Thomas Henry Simpson Memorial Institute for Medical Research of the University of Michigan.
Received for publication October 2, 1935.

("band forms")? How long do the changes persist? Do all the polymorphonuclear neutrophils show these changes?

Materials and Methods.—Eighteen young albino rats were used for this experiment. They were given the regular diet consisting of grain, cereals, lettuce, and pantry scraps.

Twenty-four-hour growths of *Staphylococcus aureus* and *Streptococcus hemolyticus* were the infecting organisms. *Staphylococcus aureus* grown on blood agar was used for Group V.* This organism was also used for those experiments in which the effect of killed bacteria was demonstrated. The doses of the organism suspended in physiologic salt solution varied from 0.5 c.c. to 4.0 c.c. The solution contained approximately 150 billion bacteria per cubic centimeter. The injections were given intraperitoneally, except the doses given for reinfection of Group I, which were given subcutaneously.

TABLE I

GROUP I

Aged six weeks
Infecting organism, *Staphylococcus aureus*
Place of injection, intraperitoneal
Place of reinjection, subcutaneous

DAY OF INFECTION	DOSE	PERCENTAGES OF (A) POLYMORPHONUCLEAR LEUCOCYTES, (B) NEUTROPHILES SHOWING BASOPHILIC GRANULES					
		ANIMAL A		ANIMAL B		NORMAL ANIMAL	
		(a)	(b)	(a)	(b)	(a)	(b)
1*	0.5 c.c.†	16	0	21	0	17.5	0
2		49	0	43	0	20.0	0
3		33	0	25	0	15.0	0
4		22	0	21	0	21.0	0
5	1.0 c.c.	31	0	15	0	19.0	0
6†		30	0	28	0	16.0	0
7		28	0	19	0	22.0	0
8		17	0	21	0	23.0	0

*Day of injection.

†Day of reinjection.

‡1 c.c. contains approximately 150 billion bacteria.

TABLE II

GROUP II

Aged six weeks
Infecting organism, *Streptococcus hemolyticus*
Place of injection, intraperitoneal

DAY OF INFECTION	DOSE	PERCENTAGES OF (A) POLYMORPHONUCLEAR LEUCOCYTES, (B) NEUTROPHILES SHOWING BASOPHILIC GRANULES					
		ANIMAL A		ANIMAL B		NORMAL ANIMAL	
		(a)	(b)	(a)	(b)	(a)	(b)
1*	1.5 c.c.‡	20.5	0	21.0	0	18.5	0
2		37.0	0	48.0	0	22.0	0
3		19.0	0	23.5	0	19.0	0
4		18.0	0	25.0	0	15.0	0
5†	1.5 c.c.‡	23.0	0	17.0	0	22.0	0
6		22.5	0	25.0	0	15.0	0
7		21.0	0	16.0	0	12.0	0

*Day of injection.

†Day of reinjection.

‡1 c.c. contains approximately 150 billion bacteria.

*See data in Tables I to V.

TABLE III

GROUP III

Aged five months
 Infecting organism, *Streptococcus hemolyticus*
 Place of injection, intraperitoneal

DAY OF INFECTION	DOSE	PERCENTAGES OF (A) POLYMORPHONUCLEAR LEUCOCYTES, (B) NEUTROPHILES SHOWING BASOPHILIC GRANULES					
		ANIMAL A		ANIMAL B		NORMAL ANIMAL	
1*	25 cc	(1)	(b)	(a)	(b)	(a)	(b)
2		17.5	0	22	0	21	0
3†	25 cc	40.0	0	9	0	15	0
4		22.5	0	21	0	24	0
5		28.0	0	16	0	17	0
6		22.5	0	19	0	1	0
		17.0	0	22	0	19	0

*Day of infection

†Day of reinfection

162 per cent consisted of 14 per cent young cells (band forms) or all the young cells and 48 per cent of the older (multilobed) forms

86 per cent consisted of 14 per cent young cells (band forms) or all the young cells and 72 per cent of the older (multilobed) forms

TABLE IV

GROUP IV

Aged five months
 Infecting organism, *Staphylococcus aureus*
 Place of injection, intraperitoneal

DAY OF INFECTION	DOSE	PERCENTAGES OF (A) POLYMORPHONUCLEAR LEUCOCYTES, (B) NEUTROPHILES SHOWING BASOPHILIC GRANULES					
		ANIMAL A		ANIMAL B		NORMAL ANIMAL	
1*	25 cc	(1)	(b)	(a)	(b)	(a)	(b)
2†	25 cc	17	0	17.0	0	19.0	0
3†	25 cc	46	0	78.5	0	24.0	0
4		77	62.5	20.0	14	16.0	0
5		34	86.5	28.0	23	18.5	0
6		32	0	21.0	0	16.0	0
				24.0	0	12.0	0

The bone marrow of Animal A did not show any basophilic granules on the fourth day of infection

*Day of infection

†Day of reinfection

162 per cent consisted of 14 per cent young cells (band forms) or all the young cells and 48 per cent of the older (multilobed) forms

86 per cent consisted of 14 per cent young cells (band forms) or all the young cells and 72 per cent of the older (multilobed) forms

Blood films were made daily. The blood was taken from the tip of the tail, spread on No. 1 cover glasses, and stained with Wright's stain by the following slightly modified method. The concentrated solution was diluted at the end of one minute according to the usual technique but after dilution with water, the mixture was allowed to stand for ten minutes. Brilliant cresyl blue stain was also used to insure a closer observation of changes in staining of the neutrophils.

Two hundred leucocytes were counted to determine the percentage of polymorphonuclear neutrophilic leucocytes present, and one hundred neutrophils were counted to obtain the percentage of cells showing changes in staining reaction.

The bone marrow was studied by making impressions of the marrow of the ribs, sternum, and femur on cover glasses by the abklatzsch or touch method and

stained with Wright's stain by the usual technic. Serum suspension films were also made and stained in the same way.

TABLE V

GROUP V

Aged twelve weeks
 Infecting organism, *Staphylococcus aureus*
 Place of injection, intraperitoneal

DAY OF INFECTION	DOSE	PERCENTAGES OF (A) POLYMORPHONUCLEAR LEUCOCYTES, (B) NEUTROPHILES SHOWING BASOPHILIC GRANULES					
		ANIMAL A**		ANIMAL B†		NORMAL ANIMAL	
		(a)	(b)	(a)	(b)	(a)	(b)
1*	4.0 c.c.	11	0	22	0	16.5	0
2*	4.0 c.c.	49	0	36	0	22.0	0
3		40	88‡	31	0	25.0	0
4				27	0	18.0	0

The bone marrow of Animal A showed heavy basophilic granules on the third day of infection.

*Day of injection.

**Animal A was injected with living organisms.

†Animal B was injected with dead organisms.

‡88 per cent consisted of 12 per cent young cells ("band forms") and 76 per cent older (multilobed forms).

The 12 young cells and 52 per cent of the older forms contained many basophilic granules and the remaining 24 per cent contained fewer granules with an affinity for methylene blue.

DISCUSSION

The data show that *Staphylococcus aureus* and *Streptococcus hemolyticus* produce similar results when injected into the rat. With both these organisms, there is an early increase in the number of polymorphonuclear neutrophilic leucocytes, but both fail to maintain this increase for any considerable length of time. A second injection of either streptococcus or staphylococcus given after the animal has recovered from a previous injection of that organism produces only a slight increase in the number of neutrophiles.

In Groups I, II, and III a single injection did not produce an appreciable basophilia of the granules. A second dose given after the effects of the first dose had worn off did not produce basophilia of the granules of the polymorphonuclear neutrophilic leucocytes.

Injections given on successive days with Groups IV and V were followed by staining changes in the polymorphonuclear neutrophiles (basophilia of the granules). With Group IV, however, the basophilia of the neutrophiles was found only in the peripheral blood, while with Group V the change extended to the bone marrow.* This change appeared twenty-four hours after the second successive injection and persisted for forty-eight hours. All the young forms ("band forms") showed the change in the staining but this was not true of all the older (multilobed) forms. At no time during the infection did all the neutrophiles show basophilia of the granules.

There was no basophilia of the granules of the neutrophiles of the blood of the rats injected with dead bacteria.

*It required larger doses and a more virulent organism to produce the change in the bone marrow.

SUMMARY AND CONCLUSION

Rats react similarly to *Staphylococcus aureus* and *Streptococcus hemolyticus* by showing an increase in the number of polymorphonuclear neutrophilic leucocytes. They recover rapidly from infection and show a relative "immunity" to the infecting organism by failure to respond to a second dose after having recovered from a previous dose of the particular organism. It is necessary to give larger doses at short intervals (successive days) to produce basophilia of the granules of the neutrophils of the peripheral blood. Larger doses of a more virulent organism are necessary to produce staining changes in the bone marrow. All the young polymorphonuclear cells ("stiff forms"), but not all the older (multilobed) forms show basophilia of the granules. Many of the neutrophils have large "toxic" granules, while the basophilic granules of some of the remaining neutrophils were scanty and interspersed among the neutrophilic granules.

It may be concluded that basophilia of the granules of the polymorphonuclear neutrophilic leucocytes may be produced experimentally in rats that the infection must be sustained for at least forty eight hours to produce this change, that the change occurs in both the younger ("band forms") and the older (multilobed) forms, that basophilia of the granules of the peripheral blood persists for forty eight hours from the time the change appears, and that larger doses, capable of overcoming the resistance of the rat, are necessary to bring about a greater affinity for methylene blue stain in the younger cells in the bone marrow.

REFERENCES

- 1 Cesaris Demel, A. Ueber die morphologischen Struktur mit den morphologischen und chromatischen Veraenderungen der Leukozyten, Virchows Arch f path Anat 195 1, 1908
- 2 Mommsen, H. Die Pathologische ("toxische") granulation der feingekorneten Leukozyten, ihre objektive Erkennung und praktische klinische Verwertung, Klin Wchnschr 42 2420, 1929
- 3 Gloor, W. Die klinische Bedeutung der Qualitativen Veraenderungen der Leukozyten, Leipzig Georg Thieme, 1929
- 4 Matis, quoted from Schilling, V. The Blood Picture and Its Clinical Significance, translated by R B H Gradwohl, St Louis, 1929, The C V Mosby Company
- 5 Rosenthal, N, and Sutro, C J. Blood Picture in Pneumonia, With Special Reference to Pathological Changes in Neutrophils, Am J Clin Path 3 181, 1933
- 6 Meranze, D, Mendell, T, and Meranze, F. Cytoplasmic Changes in the Peripheral Neutrophil as an Aid in Diagnosis and Prognosis, Am J M Sc 189 639, 1935
- 7 Sutro, C J. Changes in Circulating Leukocytes in Infection, Arch Int Med 51 747, 1933
- 8 Spaeth, E. Über die Bedeutung der Granulaveränderungen der feingekorneten Leukozyten, Ztschr f klin Med 118 406, 1931
- 9 Fitzhugh, T, Jr. The Age of the Leukocyte in Relation to Infection, J LAB & CLIN Med 17 975, 1932

THE BLOOD SUGAR LEVEL AFTER PROLONGED CARBOHYDRATE FEEDING*

M. CAROLINE HRUBETZ, NEW YORK, N. Y.

MANY hold that a major contributing cause of human diabetes is an intemperance in carbohydrate consumption.¹⁻⁷ In sharp contradistinction with man, idiopathic diabetes in domestic animals is almost unknown, and the only practical means by which experimental diabetes, which can be said to resemble diabetes mellitus, has been induced in the lower forms, involves some form of injury to the pancreas or, more precisely, to the islets.

Several years ago a research involving these and other problems was started in this laboratory upon rats in which the animals were fed the usual stock diet plus 25 per cent sucrose.

This research has progressed now to the point where four generations have been born and raised to maturity upon this diet. There has been no indication of idiopathic diabetes and but one case of marked obesity. A detailed study of the blood sugar characteristics follows.

Twenty-eight rats at the age of 100 days were placed upon the normal diet plus 25 per cent sucrose. Blood sugars were taken at intervals over a period of nineteen months, after which time it was no longer possible to obtain samples by the usual method. From these original 28 rats, four generations of offspring have been and are being followed, each generation being bled as early as size of the animal permitted. Because of other work on hand, it was not always possible to make these tests at any specified age; hence, the variations in the time intervals of the various tables. The Somogyi microblood sugar method⁸ has been used throughout the research. Tables I to VI summarize the results so far obtained.

TABLE I
ORIGINAL ANIMALS

NO. OF ANIMALS	TIME ON HIGH SUGAR DIET	BLOOD SUGAR LEVEL
50	Controls	108 mg.
28	17 days	123 mg.
28	24 days	124 mg.
28	34 days	126 mg.
28	110 days	107 mg.

During the first month on high carbohydrate, the animals showed an elevated blood sugar. After 110 days the blood sugar had reached normal and remained so throughout the 600 days they were observed.

*From the Department of Physiology, College of Physicians and Surgeons, Columbia University in the City of New York.

Received for publication, October 2, 1935.

Seven animals bled at 94 days of age showed a high sugar level but subsequent observations carried on through 500 days were all normal

All second generation animals showed normal blood sugar levels at the time of observation and throughout the 575 days observed

TABLE II
FIRST GENERATION

NO OF ANIMALS	TIME ON HIGH SUGAR DIET	BLOOD SUGAR LEVEL
50	Controls	108 mg
7	94 days	122 mg
2	150 days	102 mg
41	260 days	100 mg

TABLE III
SECOND GENERATION

NO OF ANIMALS	TIME ON HIGH SUGAR DIET	BLOOD SUGAR LEVEL
10	Controls	102 mg
6	81 days	108 mg
21	220 days	100 mg

TABLE IV
THIRD GENERATION

NO OF ANIMALS	TIME ON HIGH SUGAR DIET	BLOOD SUGAR LEVEL
50	Controls	103 mg
17	178 days	102 mg
12	240 days	107 mg

TABLE V
FOURTH GENERATION

NO OF ANIMALS	TIME ON HIGH SUGAR DIET	BLOOD SUGAR LEVEL
50	Controls	107 mg
1	80 days	92 mg
7	120 days	110 mg
7	170 days	102 mg

All third generation animals showed normal blood sugar readings throughout the 500 days they were observed

The first two readings taken on the fourth generation show a considerable deviation from each other. It is possible that these represent the extreme variations for the animals. The third and last observation made showed normal sugar level

Two years after this experiment was begun, the question arose as to whether or not the initial rise in the blood sugar level as observed with the original rats could be duplicated. Consequently 12 rats about 90 days of age were placed on the high sugar diet

TABLE VI

NO. OF ANIMALS	TIME ON HIGH SUGAR DIET	BLOOD SUGAR LEVEL
50	Controls	103 mg.
12	14 days	116 mg.
12	40 days	108 mg.
12	55 days	100 mg.

It will be seen that these rats, as did the original ones, showed an elevated blood sugar, the first few weeks after which time it returned to the normal level.

DISCUSSION

As has been shown, we have no evidence, so far, of any over-fatiguing of sugar tolerance. What is of interest to us is the initially high blood sugar following the administration of a high carbohydrate diet. In both cases, this high level returned to the normal and remained so throughout the periods observed. Just what mechanism is involved in this process of return to normal of the blood sugar level we are at present unable to say. A few of the animals showed some increased weight, but with the exception of one animal, this has not seemed significant. The question of increased liver storage as well as of increased metabolic rate might be considered. This colony is being continued with the hope that light may be thrown upon some, at least, of the various factors involved.

REFERENCES

1. Joslin, E. P.: *The Treatment of Diabetes*, ed. 3, Philadelphia, Lea and Febiger.
2. Joslin, E. P., Dublin, L. I., and Marks, H. P.: *Am. J. M. Sc.* 186: 753, 1933.
3. Stone, W. J.: *J. A. M. A.* 95: 711.
4. Editorial: *J. A. M. A.* 202, 1930.
5. Emerson, H.: Cited in *Editorial of Medical News* 83: 2099.
6. Nicoll, M., Jr.: From Health talk broadcast, Station WGY, Schenectady, November 15.
7. Wendt, L. F. C., and Peck, F. B.: *Am. J. M. Sc.* 181: 52, 1931.
8. Somogyi, M.: *J. Biol. Chem.* 70: 599, 1926.

THE RELATION OF THE NONFILAMENT AND FILAMENT COUNTS DURING EXCITEMENT*

H L KATZ, PH D, AND L B NICE, PH D, COLUMBUS, OHIO

A VARIETY of conditions may cause a deflection of the nonfilament and filament neutrophile counts. Danzer (1930) observed that the destruction and absorption of various tissues in vivo is followed by a deflection of the count to the left. He states "Extensive destruction of tissues in vivo, from whatever cause, results in the liberation into the blood stream of substances which stimulate the leucopoietic centers." Katz and Nice (1934) in their study of the chemical and cytologic changes in the blood during excited states found a rapid breakdown of tissue products during states of anger, fear, and pain. This was manifested by an increase in the nitrogenous constituents of the blood during these states and was accompanied by emotional leucopenia in the rabbit. Recovery to the normal neutrophile level took place within fifty or sixty minutes after the excited period.

In this investigation, we have studied the effect of the products of tissue breakdown during excitement upon the bone marrow activity as shown by the number of new neutrophiles set free into the blood stream, the cause of the emotional leucopenia in our rabbits, and the effect of excitement upon the relation of the number of the nonfilament to the filament cells.

Procedure—Healthy adult rabbits which were accustomed to being handled were used in these experiments. A drop of blood was obtained by puncturing an ear vein and cover slip smears were made and stained with Wright's stain.

The normal nonfilament and filament counts were obtained at two consecutive thirty minute intervals, after which the animal was excited by the method previously described from this laboratory. Blood smears were made immediately following the excitement and thirty and sixty minutes, respectively, after this period. One hundred neutrophiles were enumerated in each count.

We have followed the Fairley, St. Clair, and Reisinger (1930) modification of the Cooke and Ponder method in making these counts. In this modification the neutrophiles are divided into two groups: nonfilament (young cells) and filament (old cells). In the young neutrophiles, the nucleus is unsegmented, while in the filament group, fine threads of chromatin material connect two or more segments of the nucleus. We adhered closely to the dictum of Ponder, "If there is any band of nuclear material connecting the different parts of a nucleus, that nucleus for the purpose of a count cannot be said to be divided."

RESULTS

Table I shows the trend of the nonfilament count before, during, and after excitement. These counts averaged 20.8 in the first normal, 20.5 in the second,

*From the Department of Physiology, The Ohio State University.
Received for publication October 2, 1935.

Group	No. of rabbits	No. of neutrophils per field			
		Before	During	After	Control
1	5	17.4	17.9	18.1	18.6
2	5	17.9	18.1	18.6	19.2
3	5	18.1	18.6	19.2	19.4
4	5	18.6	19.2	19.4	19.2
5	5	19.2	19.4	19.2	19.2

The results of the present study of the effect of emotional excitement on the number of neutrophils in the blood of rabbits are shown in the table of experimental results. In the control group the average was 17.4, 17.9, 18.1, 18.6, and 18.6, while in the emotional excitement group were 19.6, 19.2, 19.4, 19.2, and 19.2.

The results show that the relation of the number of nonfilamentous to the filamentous cells is not altered during excitement. During the recovery from the excited state, this relation is also maintained, indicating that excitement does not produce an immediate stimulation of bone marrow activity, nor is recovery for a period of an hour characterized by an increased number of neutrophils.

SUMMARY

The products of tissue breakdown which are liberated into the blood stream during excitement (anger, fear, and pain) did not deflect the relation of the non-filamentous and filamentous neutrophils.

The emotional leucopenia in our rabbits seems to be due to the enmeshing of the neutrophils in the peripheral capillaries and tissues.

REFERENCES

Andrews, M.: Quoted, J. Exper. Physiol. 20: 111, 1930.
 Farley, D. L., H. Chubb, H., and Rensinger, J. A.: Am. J. M. Sc. 180: 336, 1930.
 Kutz, H. L., and Nee, L. R.: Am. J. Physiol. 107: 709, 1934.
 Shep, L. H., and Kutz, H. L.: Am. J. Physiol. 109: 80, 1934.

A SIMPLE TREATMENT FOR PSORIASIS*

JOSEPH KRATKA, JR., M.D., AUGUSTA, GA

PSORIASIS is a disease of unknown etiology. It has no particular relation to age, sex, color, race, nor diet. It is not transmitted by the closest family contacts. It is not hereditary. One hundred years ago, as "scaly tetter," it had an extensive pharmacopea. Recent curative agents have been sought in salvarsan, colloidal gold, intramuscular injections of the patient's own blood, sex hormones, typhoid vaccine and chaulmoogia oil. Cause and effect are confused in the study of psoriasis by the so-called "spontaneous cures," associated with bed rest, recovery from other diseases, vacations, change of diet.

A commonly observed fact in the South concerning this disease is that it generally clears up to some extent in the summer sun. This led the author to the hypothesis that it might be cured with viosterol. A trial test was made of the hypothesis. A patient with a case of ten years' standing, continuous duration, was put on a routine treatment of viosterol—two gelatin capsules containing 3 mmms each of haliver oil with viosterol, daily. Within sixty days from the beginning of the test, the skin of this patient was entirely clear.

Two selected cases were then tested: one, a nurse at the University Hospital, the other, a colored female patient from the Dermatological Clinic of the University. The two cases were admirable for the test. The first was of only three weeks' duration, the second had run a course of thirty years.

The first case was that of a young, well-nourished white girl who had recently entered the nursing class of the Hospital. About three weeks before our test began, she had "broken out" with a severe psoriasis, showing the typical lesions on arms, legs, and back, with even a few scaly patches on her forehead. She was put on viosterol, two capsules per day. This was continued from February 11 through March and the early part of April, but her condition did not materially improve. Her arms were so spotted that she had to be put to night duty in the wards. For the first month and a half she had no other treatment than the viosterol. Later she tried various standard salves and patented ointments. She did not improve.

In May (20, 1935) the viosterol treatment was increased to a massive dose of ten capsules per day for ten days. At 2,000 units of vitamin D per capsule, this would give a total of 200,000 units for the period. A ten-day rest period was interpolated and the massive dose repeated on June 11. Improvement has been slow, but now she has only a few small inconspicuous patches on the elbows and a few spots on her legs. "Maintenance doses" of two capsules per day are being continued.

The second case was that of a colored female, aged forty-nine years. She has had the disease, according to her own statement, for "thirty years." She

*From the University of Georgia School of Medicine.
Received for publication October 20, 1935.

THE CAUSE AND SIGNIFICANCE OF THE ELECTRONEGATIVITY OF ACTIVE LIVING TISSUE*

W. E. BURGE, O. S. ORTH, H. W. NEILD, R. KROUSE, AND G. C. WICKWIRE,
URBANA, ILL.

INJURY to any part of the organism initiates chemical changes which render the injured portion electronegative to the uninjured. Activity likewise initiates chemical changes which cause the active portion to become electronegative to the inactive. The active and injured area corresponds to the negative pole and the inactive and sound part to the positive pole of a battery, and if these two parts or poles be connected an electric current will flow just as is the case when the poles of a battery are connected. Chemical change is the cause of the electric current from a battery, and it is undoubtedly the cause of the action and demarcation currents in living tissue.

ELECTRONEGATIVITY IN INJURED AND CONTRACTING MUSCLE

The following experiments were carried out in an attempt to determine what chemical changes are brought about, by injuring a muscle, that cause the injured area to become electronegative. A gastrocnemius muscle of the leg of a medium-sized frog was carefully removed, skinned, and cut transversely near one end. A pair of nonpolarizable electrodes were prepared and connected with a galvanometer. When the cut or injured end of the muscle was placed against one of the electrodes and the sound surface against the other (see Fig. 1), the galvanometer registered a flow of current of two to four microamperes from the sound surface to the injured end of the muscle. The application of a small amount of a one molar solution of calcium chloride to the cut or injured end of the muscle immediately abolished the electronegativity and the demarcation current, while the subsequent application of a small amount of a 0.05 molar solution of phosphoric acid or disodium phosphate promptly restored the electronegativity and the demarcation current. This observation was confirmed with the use of a number of muscles. It was also found that the application of a one molar solution of barium chloride was as effective as the calcium chloride in abolishing the electronegativity of the injured end of the muscle and the demarcation current, while the application of a one molar solution of magnesium chloride was less effective. The use of weaker solutions of these salts was found to be effective, but the action was slower. The muscles of turtles were also used, and the results were essentially the same as those obtained with the use of frogs' muscles.

Pieces of ammonium molybdate paper were placed on the cut ends of frogs' muscles and moistened with the muscle juices. Upon removal and drying

*From the Department of Physiology, University of Illinois.
Received for publication, November 11, 1935.

of these pieces of paper at 65° C., they turned distinctly yellow, thus showing the presence of phosphate on the injured ends of the muscles. It is assumed that the application of calcium chloride as well as barium chloride abolished the electronegativity and the demarcation current by combining with the electronegative phosphate ions at the injured end of the muscle to precipitate insoluble and non-ionized calcium and barium phosphate. Magnesium chloride was less effective because the magnesium phosphate formed was more soluble and more highly ionized than either the calcium or barium phosphate. Since the removal of the electronegative phosphate ions abolished the electronegativity of injured muscle, and the restoring of these ions restored it, the conclusion is drawn that the electronegativity of injured muscle is due to the electronegative phosphate ions.

The active or contracting portion of a muscle, like the injured area, is electronegative to the noncontracting or resting portion. The contracted area

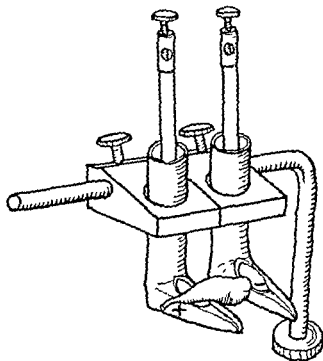


Fig. 1.—Shows injured electronegative end of a muscle against one nonpolarizable electrode and the uninjured electropositive surface against the other.

of the beating heart is electronegative to the resting portion. The phosphorus compounds, creatinphosphate and adenylypyrophosphate, occur in muscle, and it is known that these two compounds are broken down during muscular contraction with the formation of phosphoric acid and liberation of energy for the muscular contraction.¹ The electronegativity of the wave of muscular contraction that sweeps over a muscle upon stimulation like that of the injured area is attributed to the electronegative phosphate ions, arising from the hydrolysis of the creatinphosphate and adenylypyrophosphate during contraction.

The electronegativity produced by mild stimulation is transitory, passes over the muscle and disappears, while that set up by an injury is local and persistent. A very strong stimulus applied to a muscle, sufficiently strong to be injurious, produces, in addition to a transitory wave of electronegativity, a state of persistent and local electronegativity at the site of stimulation. Hence, contracted muscle may be looked upon as slightly injured muscle, and the effect of injury and stimulation is the same; namely, to liberate electro-

negative phosphate ions, and these ions in turn are responsible for the electronegativity of both injured muscle and the wave of muscular contraction.

ELECTRONEGATIVITY IN THE PRODUCTION OF ARTERIOSCLEROSIS

The carotid arteries of an anesthetized dog were exposed, and with the use of a galvanometer and platinum wire electrodes, the electropotential of these vessels was determined. It was found that when two uninjured parts of the carotid artery were connected with the micro-ammeter practically no current flowed, but when an area, either of the exterior or interior of the artery was injured, this area became electronegative to the uninjured, and this electronegativity, like that of the injured muscle, is attributed to the electronegative phosphate ions. The deposition of calcium phosphate in the walls of the arteries to produce calcification or hardening of these vessels is attributed to the combination of the electronegative phosphate ions at the site of the injury with the calcium in the circulating blood. This mechanism of calcification offers an

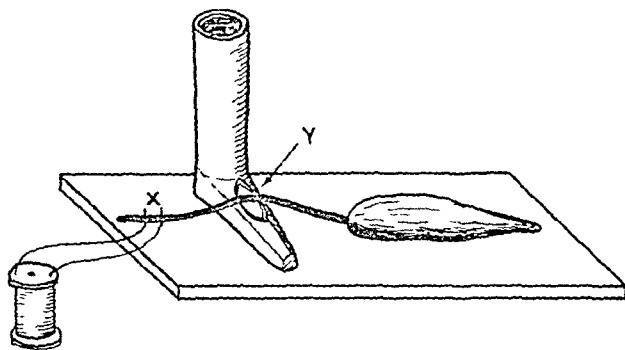


Fig. 2.—Calcium chloride applied to the nerve at "y" blocked the electronegative wave set up by the stimulation of the nerve at "x" and prevented the muscle from contracting.

explanation for the fact that the calcium deposited in the walls of the arteries is in the form of phosphate, that this deposition begins in the intima and is initiated by an injury resulting from infection, strenuous work, etc.

ELECTRONEGATIVITY IN BRAIN AND NERVE ACTIVITY

The brain of an anesthetized dog was exposed and a small area injured. With the use of a galvanometer, it was found that this injured area was electronegative to the uninjured surface or cortex of the brain, and the demarcation current was around 0.5 microamperes. The application of a few drops of 0.1 molar solution of calcium chloride to the damaged brain tissue caused the immediate disappearance of the electronegativity and demarcation current and the subsequent application of a small amount of 0.05 molar solution of phosphoric acid promptly restored the electronegativity and demarcation current. This observation was confirmed with the use of a number of dogs. The use of ammonium molybdate paper showed the presence of phosphate on the injured area of the brain. Hence, the electronegativity of the damaged brain tissue, like injured muscular tissue, is attributed to the electronegative phosphate ions.

The gastrocnemius muscle with its nerve attached was removed from the leg of a frog and prepared for stimulation (see Fig. 2). When the nerve was stimulated, waves of electronegativity were set up in the nerve, and these passed to the muscle causing waves of electronegativity and contraction in the muscle. A small amount of a one molar solution of calcium chloride was applied to the nerve between the point of stimulation and the muscle, and it was found that the muscle would still contract upon stimulation of the nerve. The sheath of the nerve was then broken between the point of stimulation and the muscle with the use of a fine needle, and the stimulation of the nerve still caused the muscle to contract. But upon the application of a 0.5 molar solution of calcium chloride to the nerve where the sheath was broken, stimulation of the nerve failed to elicit a contraction of the muscle. This observation was confirmed with the use of several nerve muscle preparations.



were connected with an inductorium preparatory for stimulation, and those on the sciatic nerve were connected with a galvanometer. Stimulation of the motor area of the brain set up waves of electronegativity or nerve impulses which passed down the spinal cord out over the motor nerves to the muscles and caused them to contract with a resulting kick of the foot. The electronegative waves that passed over the nerve were detected with a galvanometer. The electrodes were removed from the nerve and placed on the muscle, and the electronegative waves that passed over the muscle were also detected when the motor area was stimulated. The application of a one molar solution of calcium chloride to the sciatic nerve where its sheath had been broken was found to block these waves of electronegativity, and the stimulation of the motor area of the brain no longer caused a contraction of the muscles of the leg. This observation was confirmed with the use of several frogs.

The method of procedure of the preceding experiment was then reversed. The galvanometer was connected to the electrodes on the brain and the inductorium to the electrodes on the sciatic nerve. The object of the experiment was to determine if the stimulation of the sciatic nerve would produce any change in the electropotential of the cortex of the brain and if electronegative phosphate ions were involved in the production of this electrical change.

So far as stimulation of sensory nerves going to the brain producing a change in the electropotential of the brain is concerned, many experiments are reported in the literature, showing that this can be done. Bartley and Bishop² found that the stimulation of the nerves going from the eyes to the brain produced a change in the electropotential of the center for vision, the area of the cortex of the brain to which the nerves from the eyes run. Lorente de No³ showed that the stimulation of the nerves to the muscles of the eye changed the electropotential of the medulla, the portion of the brain where these nerves originate. Caton⁴ found that there was normally a continuous fluctuation in the electropotential of the cortex or surface of the brain, resulting presumably from cortical activity or the inflow of nerve impulses over sensory nerves from the outlying parts of the body. With the use of the oscillograph, much work is being done at this time on the electrical changes that occur in the brain.⁵ What these electrical changes signify or what their relation to cortical activity is or their cause so far as we are aware is unknown. However, it would seem reasonable to assume that, during brain activity, the area or areas of the cortex involved would become electronegative like all other active tissue, and our observations suggest that the cause of this electronegativity is the electronegative phosphate ions. Phosphorus is certainly very essential for muscular activity, and it may be equally as essential for brain activity. So there may be after all some scientific basis for the view formerly held that phosphorus rich foods promote mental activity.

ELECTRONEGATIVITY IN AGEING

Calcium salts are normally deposited, particularly during the period of growth, in the bony tissues to give rise to the hardness and rigidity of the mature bone. With advance in age, calcium salts are also deposited in certain of the soft tissues of the body. The crystalline lens of the eye gradually loses

phate ions with the electropositive calcium ions in the liquids of the body to precipitate insoluble and non-ionized calcium phosphate at the site of the injury.

Modern medical science has prolonged the average span of life by improving conditions so that more people live to be older, but the upper age limit still remains unchanged. The fact that the doctor lives no longer than his patient is evidence that medical science has done nothing toward solving the problem of senescence. If ageing is essentially a process of calcification of the soft tissues, then senility is an incurable disease, and the prolonging of the upper age limit is an impossibility. For life implies activity with resulting injury, liberation of electronegative phosphate ions and calcification. Hence, with the increase in the strain and activity of our modern civilization, the upper age limit is more likely to be lowered than raised.

SUMMARY AND CONCLUSIONS

1. Since the removal of the electronegative phosphate ions abolishes the electronegativity of injured muscle, and the restoring of these ions restores it, the conclusion is drawn that the electronegativity of the injured muscle is due to the electronegative phosphate ions. The electronegativity of the wave of muscular contraction that sweeps over a muscle upon stimulation is also attributed to electronegative phosphate ions arising from the hydrolysis in the contracting muscle of creatinphosphate and adenylypyrophosphate.

2. The electronegativity of an injured area of the brain like the injured muscle can be abolished by the precipitation of the electronegative phosphate ions with calcium chloride and this electronegativity can be restored by restoring these ions. Hence, the electronegativity of the injured brain is attributed to the electronegative phosphate ions. The waves of electronegativity that pass over a nerve upon stimulation can be blocked by applying calcium chloride to the nerve where its sheath has been broken. These waves of electronegativity are considered to be due to propagated electronegative phosphate ions and the blocking by calcium is attributed to the precipitation of the phosphate as insoluble and non-ionized calcium phosphate. These observations suggest that the change produced in the electropotential of the cortex of the brain by the stimulation of sensory nerves may be due to electronegative phosphate ions, just as the change produced in the electropotential of the muscle by the stimulation of motor nerves is probably due to these electronegative phosphate ions.

3. Injury to the intima of arteries was found to render the injured area electronegative to the uninjured portion of the artery. The electronegativity of the injured intima, like the injured skeletal muscle and brain, is attributed to electronegative phosphate ions. It is assumed that the calcification of the arteries is due to the combination of the electronegative phosphate ions at the site of the injured intima with the electropositive calcium ions of the circulating blood. This mechanism of calcification accounts for the fact that the calcium deposit in the walls of the arteries consists almost entirely of calcium phosphate, that the deposition begins in the intima and that it is initiated by an injury. The same mechanism is offered as an explanation for the gradual deposition of calcium in the crystalline lens of the eye. The injury of the lens is attributed to the absorbed short wave lengths of the spectrum.

4 Ageing is considered to be due to a gradual process of calcification of the soft tissues particularly the arteries with resulting impairment of function. Electronegative phosphate ions are liberated as a result of the stress and strain of the activities of life and subsequent calcification is attributed to the reaction of the electronegative ions with the electropositive calcium ions of the liquids of the body to precipitate insoluble calcium phosphate at the site of the injury.

REFERENCES

- 1 Liggelson, P., and Eggleton, G. P. A Possible New Factor in the Chemical Mechanism of Muscular Contraction, *Chem & Ind* 46 485, 1927.
- 2 Eggleton, P., and Liggelson, G. P. The Inorganic Phosphate in a Labile Form of Organic Phosphate in the Gastrocnemius of a Frog, *Biochem J* 21 190, 1927.
- 3 Fiske, C. H., and Subbrow, Y. The Nature of the "Inorganic Phosphate" in Voluntary Muscle, *Science* 68 401, 1927.
- 4 Brutley, S. H., and Bishop, G. H. The Cortical Response to Stimulation of the Optic Nerve in the Rabbit, *Am J Physiol* 103 109, 1933.
- 5 Lorente de No, R. Antidromic Impulse and Response of Mononeurons, *Am J Physiol* 112 595, 1935.
- 6 Citron, R. The Electrical Currents of the Brain, *Brit M J* 2 278, 1875.
- 7 Garceau, E. L., and Davis, H. An Amplifier, Recording System, and Stimulating Devices for the Study of Cerebral Action Currents, *Am J Physiol* 107. 300, 1934.
- 8 Jasper, H. H., and Carmichael, L. Electrical Potentials from the Intact Human Brain, *Science* 89 51, 1935.
- 9 Davis, H., Derbyshire, A. J., Lurie, M. H. and Saul, L. J. The Electrical Response of the Cochlea, *Am J Physiol* 107 311, 1934.
- 10 Bishop, G. H. Fiber Groups in the Optic Nerve, *Am J Physiol* 106 460, 1930.
- 11 Blair, E. A., and Erlanger, J. A Comparison of the Characteristics of Axons Through Their Individual Electrical Responses, *Am J Physiol* 106. 524, 1933.
- 12 Adrian, I. D. and Buyterdyk, F. J. J. Potential Changes in the Isolated Brain Stem of the Goldfish, *J Physiol* 71 121, 1931.
- 13 Burge, W. E. Analysis of the Normal and the Cataractous Lens, *Arch Ophth* 38 435, 1900.
- 14 Burge, W. F. The Mode of Action of Ultra Violet Radiation in Injuring Living Cells, With Special Reference to Those Constituting the Eye, *Am J Physiol* 39 330, 1916.
- 15 Ham, A. W. Mechanism of Calcification in the Heart and Aorta in Hypervitaminosis D, *Arch Path* 14 613, 1932.
- 16 Thomson, D. L., and Colbp, J. B. The Parathyroid Glands, *Physiol Rev* 12 309, 1932.
- 17 Kay, H. D. Phosphatase in Growth and Disease of Bone, *Physiol Rev* 12 384, 1932.
- 18 Burr, D. P. Pathological Calcification, *Physiol Rev* 12 593, 1932.
- 19 Hofmeister, F. Über Ablagerung und Resorption von Kalksalzen in den Geweben, *Ergebn d Physiol* 10 429, 1910.
- 20 Kleinmann, H. Experimentelle Verkalkung durch Zuführung von Kalksalzen, *Biochem Ztschr* 196 161, 1928.
- 21 Klotz, O. The Process of Pathological Calcification, *J Exper Med* 7 633, 1905.
- 22 Wells, H. G. Metastatic Calcification, *Arch Int Med* 7 721, 1911.
- 23 Robinson, R. The Possible Significance of Hexosephosphoric Esters in Ossification, *Biochem J* 17 286, 1923.
- 24 Hueper, W. Metastatic Calcifications in the Organs of the Dog After Injections of Parathyroid Extract, *Arch Path* 3 14, 1927.

SOME OBSERVATIONS ON GASTRIC ANACIDITY IN RELATION TO GASTRODUODENAL AND COLONIC FLORAS AND AN ASSOCIATED ANEMIA*

JOHN C. TORREY, PH.D., AND MICHAEL LAKE, M.D., NEW YORK, N. Y.

IT IS well known that ordinarily the recurring acid secretion of the stomach presents, directly or indirectly, an effective barrier to the further progress of living ingested bacteria. The content of the normal resting stomach, three to four hours after a meal, is usually sterile although great numbers of bacteria carried in the secretions of the nasal and oral cavities are constantly swallowed together with those in food. The flora of the duodenum¹⁻⁴ under normal conditions has also generally been reported as scanty and largely confined to such types as enterococcus, staphylococci, sarcinae with rarely coliform organisms. According to Van der Reis,⁵ Goldman,⁶ and others, this scarcity of bacteria continues into the jejunum to a distance up to 25 to 80 inches. This flora seems also to be an obligate and persistent type,⁵ and not appreciably modified by many bacteria richly varied in type which are continually entering the stomach with swallowed material. On the other hand, with a partial or complete failure of gastric acidity, a rich flora of a mixed buccal and cecal type tends to establish itself.⁷ The experimental results of Arnold and Brody⁸ indicated that a persistent alkalinity of the duodenum due to the absence in gastric anacidity of the recurrent waves of acidity with normal gastric functioning, and the consequent functional failure of the self-disinfective mechanism of the upper part of the normal small intestine¹⁷ are the factors of primary importance in permitting a bacterial upgrowth from the cecum and thus establishing the rich and varied duodenal flora frequently associated with gastric anacidity.

The observations reported here were carried out primarily to determine whether with the stomach eliminated as a defense mechanism because of failure of acid secretion, the duodenal flora is quantitatively or qualitatively different from the gastric in those cases uncomplicated by colonic flora upgrowth. Do the bacteria swallowed with the oral secretions find their way freely and in a viable condition into the small intestine? If not, to what degree are oral bacteria eliminated in the duodenum, and what types are particularly affected? Although there have been numerous reports on the character of the duodenal flora in the absence of gastric acidity, and it has been shown by Kopeloff⁹ and others that the gastric flora under conditions of subnormal acidity is essentially the same as that of the saliva, the relation in types and numbers of viable bacteria of the duodenal to the gastric flora and of the colonic flora to the duodenal, under such circumstances, has apparently not been studied in any great detail. Enquiry

*From the Department of Public Health and Preventive Medicine, and the Department of Medicine (Gastro-Enterology Clinic), Cornell Medical College.

Received for publication, November 6, 1935.

was also made into possible correlations of degrees of anemia and types of duodenal flora and into the constancy in type of duodenal floras.

The patients investigated, all adults, were in attendance at the Cornell Gastro-Enterology Clinic. All 26 cases had given negative tests for free acid in the gastric content after an Ewald test but had not been given the histamine test for complete achlorhydria. Hence, they are all grouped as gastric anacidity cases.

METHODS

Specimens from each patient for laboratory examination included gastric content, duodenal content, stool and often oral secretions. The patients were instructed to take 1 gr. tablet of phenolphthalein the night before, to brush teeth thoroughly on arising and to present themselves at the office with a stool specimen at about 9:00 A.M. after a 14- to 15-hour fast. In a number of the cases, well-mixed saliva specimens were then collected, and in all, the throat and mouth were thoroughly treated with an antiseptic solution by gargling and rinsing followed by sterile water. A freshly sterilized duodenal tube was then swallowed and permitted to pass to the greater curvature of the stomach. In obtaining specimens from 15 of the cases, the bucket of the tube was covered with a collodion membrane, using the technic of Freeman and Miller¹⁰ with the purpose of preventing contamination. After the bucket had reached the stomach position, the membrane was ruptured by air pressure, the content aspirated, the stomach thoroughly washed with sterile water, and the tube withdrawn. Another tube with covered bucket was then swallowed and the passage into the duodenum assisted by swallowing four ounces of sterile water. When fluoroscopy had shown the bucket in the third part of the duodenum, the membrane was ruptured and the duodenal content aspirated. This technic had the theoretical advantage of minimizing contamination of the duodenal specimen with the gastric flora, but on the other hand was time-consuming and trying to the patient. Experience, in fact, indicated that if the stomach after obtaining the specimen, was thoroughly washed with sterile water, a weak antiseptic solution, and then sterile water until the returning solution was clear, the same tube could then be passed into the duodenum with as little likelihood of contamination by stomach content as with the two-tube method. This single-tube method was used in Cases 16 to 25, and the findings were essentially like those obtained with the two-tube method (Cases 1 to 14). The factor of swallowed saliva could not be controlled entirely under either technic, but the findings indicated it did not seriously affect the purpose of the experiments.

Reaction and cultural examinations were started shortly after obtaining the specimens. These were kept chilled in the interim. The methods employed have been described in detail elsewhere.¹¹ For reaction determination, 0.1 c.c. of gastric or duodenal material was added to 1 c.c. of diluted bromthymol blue and also phenol red and comparison made with known standards. For aerobic organisms bromcresol purple lactose agar and blood agar plates were surface seeded, for the lactic acid group poured whey agar plates were made and for *Cl. welchii* and other spore-bearing anaerobe determinations cooked minced meat tubes were seeded with graded dilutions with subsequent plating of the growth.

The putrefactive propensity of the whole flora of the stool was estimated according to the method of Leonard and Feirer¹² slightly modified. This method, however, serves as a quantitative gauge solely of the activity of proteolytic spore-bearing anaerobes.

REACTION OF SPECIMENS

In a group of 21 of these gastric anacidity cases, the average reaction of the gastric content was pH 7.2 with ranges from pH 6.8 to 8.0 and for the duodenal content, pH 7.3 with ranges from 6.8 to 7.9. This finding for the duodenum is very close to that reported by Knott¹³ for 15 achlorhydria cases, i.e., an average of pH 7.4 with a minimum of 7.0 and a maximum of 7.8. The nature of the flora, according to our findings, seemed to exert little, if any, influence on the reaction, as in 10 cases with numerous coliform types in the gastric and duodenal content the average findings were 7.15 and 7.3, respectively, whereas in 10 cases with weakly acid-producing streptococci and no *B. coli* the averages were 7.3 for the gastric and 7.4 for the duodenal contents.

TYPES OF FLORA IN GASTRIC AND DUODENAL CONTENT

It is well known that in the absence of gastric acidity the flora of the gastric content is much the same as that of the mouth secretions (streptococci of various types, *L. acidophilus*, yeasts, gram-negative cocci). In addition, there may be representatives of the colonic flora (*B. coli*, *Cl. welchii*, and other spore-bearing anaerobes, enterococci). In five cases, second examinations of the gastric content were made at an interval ranging from seven to seventy-nine days from the first. The findings indicated a marked constancy and persistence of bacterial types in the stomach content, in spite of the fact that two patients had had acid treatment in the interim. Quantitatively the gastric flora is generally rich; in this series it was so in 22 cases with relatively few bacteria in only 4 cases. In 15 cases or 57.7 per cent, there were numerous coliform types in the gastric flora, and hence evidence of the upgrowth of the colonic flora: *B. coli communis* in 3, *B. coli communior* in 4, *B. lactici acidii* in 2, *B. aerogenes* in 1, mixtures of *communis* and *lactici acidii* in 1, and *communior* and *aerogenes* in 1. In 12 (80 per cent) instances, the same *B. coli* types were present in the gastric and duodenal content as in the stool, and in 3 (20 per cent) they were different. In 14 of the 26 cases, staphylococci were found in the gastric or duodenal content; in 12, *Staphylococcus aureus*, and in 2, *Staphylococcus albus*. They were apparently generally of oral origin. *L. acidophilus* was present in the gastric content in 22 of the cases and without doubt mainly of oral origin. *Cl. welchii* was definitely identified as present in gastric and duodenal content in 4 cases and was probably present in three others. As these positive findings were in each instance associated with high *Cl. welchii* counts for the stool specimens and generally with *B. coli* in the gastric content their presence was probably due to upgrowth of the cecal flora.

In 14 or 54.5 per cent of the cases the standardized suspension of stool¹¹ was positive for *Cl. welchii* up to 10⁹ dilution, in 5 or 19.2 per cent for the 10⁵ to 10⁷ dilutions and in 7 or 26.3 per cent to only 10⁴ to 10³ dilutions; the last is no higher than the usual findings for individuals with a normal gastric acidity.

Moensel, Kahn, and Torrey¹¹ and others, in a study of the intestinal flora in pernicious anemia, have noted unusually high counts for *Cl welchii* in the stool specimens. Nye¹² later reported that equally high counts for *Cl welchii*, as revealed by spore counts and cultures of stools, were associated with gastric achylia in patients not suffering from pernicious anemia. He ascribed these high counts to the tendency of *Cl welchii* to form spores in the supposedly more alkaline intestinal environment associated with gastric achylia, and hence to assume a more readily recognized form and not to any actual increased growth. The spore estimations for stool specimens of his 5 cases of achylia gastrica, as compared with that for 6 normal individuals as given in his tabulations, indicate an average of approximately 250,000 times as many in the former as the latter. In 14 of our gastric anacidity cases (54.5 per cent), uncomplicated by pernicious anemia, the estimations for *Cl welchii* (spore stage) were approximately one million times greater than that we have found usual for stool specimens from individuals with a normal gastric acidity, and in 5 cases (19.2 per cent), the counts were from 100 to 10,000 times greater. On the other hand, in 7 cases (26.3 per cent) the counts were approximately the same as for normal individuals. In the four cases which showed *Cl welchii* in the gastric or duodenal content or both, the *Cl welchii* counts for the stools were high, in three of these cases there was evidence of upgrowth of the colonic flora (*B coli*).

There was no evidence that absence of gastric acidity was particularly conducive to putrefactive conditions in the large intestine due to spore bearing anaerobes. In 14 cases, or 56 per cent, the indications of putrefaction were absent or no more than slight, in 6, or 24.4 per cent, only moderate, and in only 5, or 20 per cent, marked. This is about what might be expected in a series not selected for anacidity. Torrey and Schwartz¹³ for 30 cases of psoriasis reported that in 77 per cent the indication of putrefaction was slight or negative in 10 per cent moderate, and in 14 per cent marked. Leonard and Fienen,¹⁴ in a series of 127 adults not selected for gastric anacidity, found no or slight evidence of putrefaction in 50 per cent, in 33 per cent no more than moderate, and in about 20 per cent marked putrefactive tendencies.

No correlation was found between high *Cl welchii* counts and evidence of putrefactive propensities of the colonic floras, but rather the contrary. In 11 or 79.9 per cent of the cases showing no more than slight evidence of putrefaction, the *Cl welchii* counts were particularly high.

THE RELATION OF THE GASTRIC TO THE DUODENAL AND COLONIC FLORAS IN THE ABSENCE OF GASTRIC ACIDITY

In Table I the findings for 11 cases of gastric anacidity are given in which a simultaneous bacterial survey was made for the mouth secretions, the gastric, the duodenal and the colonic (stool) contents with purpose of determining what differences quantitative and qualitative may appear in the duodenal as compared with the gastric flora and to what extent, as regards numbers and types mouth organisms under such conditions survive the passage through the entire gastrointestinal tract. Particular attention was given to the gram-posi-

TABLE 1

CASE	SEX AND AGE	MOUTH FLORA	GASTRIC FLORA	DUODENAL FLORA	COMPARISON OF DUODENAL WITH GASTRIC FLORA	COLONIC FLORA (STOOL)	PURGATIVE PROPENSITY
12. T.	M 29	Streptoc. (gamma) L. acidoph.	Streptoc. (gamma and alpha) Staph. aureus L. acidoph.	Streptoc. (gamma) Staph. aureus	75% decrease (Streptoc.)	B. coli Streptoc. (gamma)* Enterococcus L. acidoph. Cl. welchii B. coli (lactic acid), aerogenous Enterococcus L. acidoph. Cl. welchii	None
13. S.	M	Streptoc. (alpha primo, beta) Enterococcus Staph. aureus	Streptoc. (alpha, alpha primo, gamma) Enterococcus Staph. aureus	Streptoc. (gamma) Enterococcus	90% decrease (Streptoc. alpha and Staph.)	B. coli (lactic acid), aerogenous Enterococcus L. acidoph. Cl. welchii	Moderate
14. C.	M 43	Streptoc. (alpha and gamma) Enterococcus	Streptoc. (alpha, alpha primo, gamma) Enterococcus B. coli (communis) L. acidoph. Cl. welchii	Streptoc. (gamma) Enterococcus B. coli (communis) L. acidoph.	60% decrease (Streptoc. alpha, alpha primo)	Enterococcus Streptoc. (gamma) B. coli (communis) L. acidoph. Cl. welchii	None
15. B.	F 49	Streptoc. (alpha, gamma)	Streptoc. (alpha, alpha primo, gamma) Staph. aureus Cl. welchii	Streptoc. (alpha primo, gamma) Staph. aureus L. acidoph. Cl. welchii	90% decrease (all types except Cl. welchii)	B. coli Enterococcus L. acidoph. Cl. welchii	None
16. C.	M 54	Streptoc. (alpha, alpha primo)	Streptoc. (alpha, alpha primo) L. acidoph.	Streptoc. (alpha, gamma) Enterococcus	99% decrease (Streptoc.)	B. coli Enterococcus Cl. welchii	Slight

*Buccal organisms surviving in the colon are killed.

TABLE I.—CONT'D

CASE	SEX AND AGE	MOUTH FLORA	GASTRIC FLORA	DUODENAL FLORA	COMPARISON OF DUODENAL WITH GASTRIC FLORA	COLONIC FLORA (STOOL)	PUTREFACTIVE PROPENSITY
17 R	F 65	Streptoc (alpha, gamma), Enterococcus B coli (communior)	Streptoc (alpha) B coli (lactici acid) L acidoph	Streptoc (alpha, alpha prime) Enterococcus B coli (lactici acid) L acidoph	50% decrease (Streptoc)	B coli (lactici acid) Enterococcus L acidoph Staph aureus Cl welchii	Moderate
18 O	F 32	Streptoc (alpha, gamma) Enterococcus	Streptoc (alpha, gamma) B coli (communis) Enterococcus	Streptoc (alpha, gamma) B coli (communior) Enterococcus	No decrease No change in flora	B coli (communis, comm minor, lactici acid) Enterococcus Cl welchii	None
19 M	F 27	Streptoc (gamma) Enterococcus	Streptoc (alpha, gamma) Enterococcus Staph albus B coli (communis)	Streptoc (gamma) Staph aureus B coli (communis, comm minor) Enterococcus	80% decrease (Streptoc)	B coli (communis, comm minor, lactici acid) B aerogenes Enterococcus Cl welchii	None
23 M	M 69	Streptoc (gamma) Enterococcus	Streptoc (alpha) Enterococcus	streptoc (alpha, gamma) Enterococcus	No decrease	B coli Enterococcus L acidoph Cl welchii	None
25 A	F 48	Streptoc (alpha) Enterococcus	Streptoc (alpha) Staph aureus Enterococcus B mucosus	Enterococcus B mucosus	99% decrease (Streptoc, Staph)	B coli B mucosus Streptoc Cl welchii	None
26 F	M 55	Streptoc (gamma) Enterococcus	Enterococcus B coli (communior) L acidoph	Streptoc (gamma) Enterococcus B coli (communior)	50% decrease (Streptoc)	Streptoc (gamma) Enterococcus B coli (communior) Cl welchii	Slight

five organisms (chain-forming streptococcus and staphylococcus), as they were most likely to be of oral origin. In 9 of these 11 cases (81.8 per cent), there was noted a very marked quantitative decrease when the duodenal flora was compared with the gastric. This was due to the partial or complete elimination of streptococcal types (alpha, alpha prime and gamma) of oral origin, pneumococcus when present, and to some extent staphylococci. Including the whole series of 25 cases, in 18 (72 per cent) the duodenal bacterial counts were 50 to 90 per cent lower than the gastric and in 9 (36 per cent) the reduction amounted to 90 per cent or more. In 7 cases (28 per cent), there was no quantitative difference.

In a few cases, the duodenal flora was entirely different from that of the stomach. When *B. coli* was present in the stomach, it was found in equal or greater numbers in the duodenum and almost invariably the same type. The most marked differences in the gastric and duodenal floras were in streptococcal types. Those of the gastric content were, as might be expected, practically identical with those of the buccal secretions, but in most instances the alpha and alpha prime chain-forming types, prominent in the gastric flora, were found greatly reduced or eliminated in the duodenal flora. For identification, dependence was placed on colony type, blood agar changes, morphology, and particularly fermentations. Among the 25 cases in 7 instances, the streptococci of the duodenum were entirely different from the gastric types, in 16 cases the differences were marked but not complete, and in only 2 cases were the streptococcal types exactly the same. In view of the fact that in most instances there were from 75 to 90 per cent fewer viable streptococci in the duodenal than the gastric content, and that on the other hand the floras of the mouth and gastric content were practically identical in the numbers and types of streptococci, the findings indicate, first, that the duodenal specimens were not seriously contaminated because of the technic employed, and second, that even in the absence of gastric acidity with its inherent bactericidal action and its reported activating influence on the autodisinfecting mechanism of the duodeno-jejunal mucous membrane (Rolly and Liebermeister,¹⁷ 1905, Arnold and Brody,¹⁸ 1926), the conditions in the duodenum still present a fairly effective barrier to the majority of buccal streptococci.

In Table I, the surviving oral streptococcal types are in italics. Only two types frequent in the mouth seemed to survive passage to the rectum with a fair degree of regularity. One type traced through in 5 of the 11 cases is designated as enterococcus on the basis of colony formation, morphology, and the fermentation of esculin. It differed, however, from the typical enterococcus in failure to grow in 2 per cent bile broth and in seldom splitting mannitol. The second type was a chain-forming gamma streptococcus of distinctive cultural characteristics. This was traced through in 3 of the 11 cases. Both of these types were raffinose positive. Aside from *L. acidophilus* and yeasts, they appeared to be the only mouth organisms capable of survival. The types constantly eliminated were the long chain alpha and alpha prime streptococci of the mouth, staphylococci, and also beta streptococci in the one case in which they were noted. The total 25 cases showed viable buccal streptococci in the rectal content in about

50 per cent. They were usually, however, greatly outnumbered by typical intestinal enterococci. It may be concluded then, that even with the barrier of gastric acidity removed, other factors selectively operative in the duodenal region and bacterial antibiosis at lower levels serve to prevent the free passage of most oral streptococci through the small and large intestine.

Although without doubt the maintenance of the scanty flora of the upper small intestine is primarily correlated with the recurrent waves of the normal gastric acid secretions as Arnold and Brody state, persistent alkalinity of the duodenal content is apparently not the only factor which determines the presence or absence of a colonic type of flora at that level. In this series of 26 cases, there occurred an upgrowth of the colonic flora in 15 (57.7 per cent), and the same types of *B. coli* were recovered from the stomach and duodenum as for the stool in 80 per cent, but the establishment of this coliform flora was not particularly linked with the greater degrees of duodenal alkalinity. Thus in 10 cases with a coliform flora in the duodenal region there were 5 with a duodenal alkalinity of pH 7.4 to 7.8, whereas among 11 cases in which there was no such evidence of upgrowth of the colonic flora there were 6 with an alkalinity of pH 7.4 to 7.9.

THE CHARACTER OF THE GASTRODUODENAL FLORA IN RELATION TO BLOOD FINDINGS

The most frequent blood picture shown by these achlorhydria cases was a moderate secondary anemia with a color index somewhat below 1.0, a leucopenia and a relative decrease of the polymorphonuclear leucocytes. There were no examples of pernicious anemia or of typical simple achlorhydric or achylie anemia. In simple achylie anemia, the color index is almost always below 0.6, according to the findings of Menlengrucht.¹⁸ Rosenthal and Abel,¹⁹ in a series of 43 cases of simple achlorhydric anemia, reported the color index as between 0.5 and 0.7, but in some instances below 0.5. Of our series, in the 19 cases in which blood examinations were made, there were 9 with a red cell count below 4,000,000 and a hemoglobin finding below 70. None of the cases, however, showed the low color index of achlorhydric anemia, the range being between 8.0 and 1.08. In Tables II and III respectively, there have been grouped the 9 cases showing some degree of anemia, and six cases with a practically normal blood picture, together with the bacterial findings for gastric and duodenal content and the *Cl. welchii* counts for the colonic (stool) flora in order to disclose any possible relation between abnormal distribution of bacteria in the gastrointestinal tract, as regards numbers and types, and the blood findings.

Among the 15 cases detailed in these two tables there were 6 or 40 per cent, showing upgrowth of the colonic flora to the gastroduodenal region as evidenced by the presence of *B. coli* in varying numbers in specimens from this locality. The three cases (7, 9, 24) showing *B. aerogenes* in small numbers were not considered as showing a definite upgrowth. Among these six cases with upgrowth of the colonic flora to the gastroduodenal region, there were four with a moderate degree of anemia and two with a practically normal blood picture. On the other hand among the nine with no upgrowths of the colonic flora, at least to the duodenum, there were five with a moderate degree of

The United States Department of Agriculture² lists several of the chemical compounds present in the spray, as calcium pentasulphide, calcium tetrasulphide, calcium thiosulphate, sulphur, and calcium sulphite. The concentrate generally retails for about twenty-five cents a gallon.*

SUMMARY

A low cost, efficient dip is described. It has been of great value in curing and preventing mange in laboratory dogs.

REFERENCES

1. U. S. Dept. Agric., Dept. Cir. No. 338.
2. U. S. Dept. Agric., Farmers Bull. No. 1287.

*Ergene Distributing Company, Leesville, S. C.

A NEW MICROREACTION FOR THE SERODIAGNOSIS OF SYPHILIS†

AN ADAPTATION OF THE KAHN STANDARD ANTIGEN

ARTURO R. CASILLI, M.D., F.A.C.P., ELIZABETH, N. J.

ELIMINATING the hemolytic system simplified the serodiagnosis of syphilis by substituting the relatively simple flocculation and precipitation tests for the complicated complement fixation method. Although flocculation tests had been attempted previously, it was the events of the Great War which stimulated German workers to reinvestigate the problem, with the result that Sachs-Georgi gave to the world one of the first practical applications of flocculation in the serodiagnosis of syphilis. The results of this test were impressive but not completely convincing, consequently many experimenters attempted to remove its imperfections. It was R. L. Kahn, however, who produced a notably simple flocculation test, which has successfully withstood critical clinical analysis. The Kahn test is generally accepted to be sensitive and specific; nevertheless, the originator and numerous later investigators have aimed to increase its sensitivity.

Recent work has led to the development of various slide tests, which have gained favor with progressive serologists. The chief advantage of the slide test is the use of a smaller amount of serum in producing the microreaction. On the other hand, it must be noted that these modifications have not, on the whole, yielded more accurate results or simplified technical procedures. Besides, the antigen used for the microreaction is usually reinforced with tincture of gums or resins, thereby endangering specificity. Perhaps Kline's slide test is the most promising of its type, as it gives results equaling, and according to its author and others, surpassing those of the Kahn test. In my opinion, however, the Kline method increases technical difficulties by requiring an especially prepared antigen and particular equipment.

†Received for publication, November 6, 1935.

That the antigen, necessary for the serodiagnosis of syphilis, is theoretically nonspecific, but practically specific, is well recognized by all serologists. The reinforcement of heart lipoidal extracts with cholesterol is known to render them more sensitive. Kline in his brilliant work has probably carried the saturation of lipoids with cholesterol to the maximum degree. However, at this point, I wish to emphasize that when 6 mg of cholesterol are added to every 1 cc of lipoidal extract, cholesterolization is sufficient and safe for practical purposes, giving specific and sensitive results. The method which I propose is based on the recognition of this fact. The advantages are (1) That it requires no special equipment, (2) that it is extremely simple, (3) that the readings are clear cut, (4) that the reaction is immediate, and (5) that there is no danger of producing false precipitation or readings in the hands of the average technician. I believe that, in specificity and sensitivity, this method, at least, equals the Kahn test tube method.

TABLE I
SLIDE COMPARISON TABLE

NUMBER	KAHN	WASSERMANN	SLIDE
<i>Complete Agreement</i>			
68	+++	+++	+++
1	++	++	++
104	---	---	---
Total 173			
<i>Agreement With Kahn—Disagreement With Wass</i>			
3	+++	++	+++
4	++	---	++
2	+	---	+
1	+	AC	+
Total 10			
<i>Agreement With Wass—Disagreement With Kahn</i>			
1	+	---	---
Total 1			
<i>Disagreement With Both Wass and Kahn</i>			
1	++	++	+++
1	++	--	+++
1	+		+++
1	++		++
1	+	--	+++
1	+++	+++	++
4	+		++
1	+	+	---
Total 11			
Grand total 195			
Agreement of slide with Kahn			98.97%
Agreement of slide with Wassermann			90.77%
Agreement of Kahn with Wassermann			90.77%

Method—The antigen is prepared and titrated exactly according to the instructions given by Kahn. After the antigen salt mixture precipitate has been standing for one half hour, although ten minutes will be sufficient, the mixture is centrifuged at high speed for approximately ten minutes. This last step constitutes the first modification. The idea, however, is not new, as some years ago I read of a similar procedure in, I believe, an abstract by the *Journal*

gressive primary tuberculous complex. Seventeen cases were associated with the stage of early dissemination, 2 cases with the stage of isolated tuberculosis in organs and organ systems, and 14 cases with the stage of late generalization.

SUMMARY AND CONCLUSIONS

This paper is based upon the study of 47 patients, 39 of whom died of tuberculous leptomeningitis, 23, or 58.97 per cent, of whom were definitely free of older lesions in the brain, meninges, or adjacent bony structures. Twelve patients, or 30.76 per cent, had multiple tuberculomas, of whom only 5 seemed to have been responsible for a tuberculous leptomeningitis. In the remaining 7 patients, meningitis was apparently the result of a direct hematogenous dissemination. Of 4 cases, or 10.25 per cent, of tuberculous leptomeningitis in brains containing single older tuberculomas, only 2 cases could be attributed to these older foci in the brain. Eight of the patients did not have tuberculous leptomeningitis, and 4 of these 8 had large tuberculomas situated in the brain tissue.

In totaling the number of patients with tuberculous leptomeningitis, 32, or 82.05 per cent, seemed to have been due to a direct hematogenous spread, and in only 7 patients, or 17.95 per cent, were there indications that older lesions in the brain or meninges had caused the diffuse infection of the meninges.

REFERENCES

1. Kaufmann, E.: Kaufmann Pathology, Philadelphia, 1929, P. Blakiston's Son & Co. 3: p. 1832.
2. Austrian, C. R.: Experimental Tuberculous Meningitis, Bull. Johns Hopkins Hosp. 27: 237, 1916.
3. Beattie, J. M., and Dickson, W. E. C.: Textbook of Pathology, St. Louis, 1926, The C. V. Mosby Co., p. 995.
4. Karsner, H. T.: Human Pathology, Philadelphia, 1926, J. B. Lippincott Co., p. 923.
5. Brown, W.: Meningeal Tuberculosis, Edinburgh M. J. 42: 126, 1935.
6. Rich, A. R., and McCordock, H. A.: An Enquiry Concerning the Rôle of Allergy, Immunity, and Other Factors of Importance in the Pathogenesis of Human Tuberculosis, Bull. Johns Hopkins Hosp. 44: 273, 1929.
7. Rich, A. R., and McCordock, H. A.: The Pathogenesis of Tuberculous Meningitis, Ibid. 52: 5, 1933.
8. MacCallum, W. G.: A Textbook of Pathology, Philadelphia, 1932, W. B. Saunders Co., p. 661.
9. Boyd, W.: Pathology of Internal Diseases, Philadelphia, 1931, Lea & Febiger, p. 725.
10. MacGregor, A. R., Kirkpatrick, H. J. R., and Craig, W. S.: Meningeal Tuberculosis; Bacteriology and Pathology, Edinburgh M. J. 42: 138, 1935.
11. Stevens, H. C.: New Brain Slicer, Arch. Path. 12: 90, 1931.
12. Thalhimer, W., and Hassin, G. B.: Clinico-pathologic Notes on Solitary Tubercle of the Spinal Cord, J. Nerv. & Ment. Dis. 55: 161, 1922.
13. Jennings, F. L.: Tuberculoma of the Spinal Cord, Ann. Int. Med. 7: 1240, 1934.
14. Radmann, C.: Zur Pathogenese der Meningitis Tuberculosa, Virchow's Arch. 295: 563, 1935.
15. Nagao, K.: The Fate of India Ink Injected Into the Bloodstream, J. Infect. Dis. 27: 527, 1920.
16. Nagao, K.: The Fate of Killed Non-hemolytic Streptococci Injected Into the Blood and the Resulting Cellular Change, Ibid. 27: 330, 1920.
17. Maximow, A. H., and Bloom, W.: Textbook of Histology, Philadelphia, 1930, W. B. Saunders Co.
18. Felton, L. D., and Wegeforth, P.: The Production of Experimental Meningitis by Direct Inoculation Into the Subarachnoid Space, Monogr. Rockefeller Inst. Med. Research 12: 5, 1920.
19. Weed, L. H., Wegeforth, P., Ayer, J. B., and Felton, L. P.: The Influences of Certain Experimental Procedures Upon the Production of Meningitis by Intravenous Inoculation, Ibid. 12: 57, 1920.
20. Ayer, J. B.: Experimental Acute Hematogenous Meningitis, Ibid. 12: 113, 1920.
21. Blacklock, J. W. S.: In a communication, Edinburgh M. J. 42: 145, 1935.

- 22 Kuba, H. Pathologisch Histologische Studien über die Entstehung der tuberculösen Meningitis beim Menschen, Abstracted in the *Zentralblatt für allgemeine Pathologie* 48 399, 1935
- 23 Kment. Quoted by Rich et al. *Bull. Johns Hopkins Hosp.* 52 5, 1933
- 24 Ariel, M. B., and Ssolowjew, A. Zur Frage der Ausbreitung in den weichen Hirn und Rückenmarkshäuten bei intravenöser Einführung

NOTE.—Since this paper was submitted for publication my attention was called to a paper written by Ariel and Ssolowjew²⁴ who have shown that after repeated intravenous injections of India ink they found the pia mater to contain a few isolated cells filled with India ink. The same type of cell was found in the subarachnoid space, in the lumen of the meningeal veins and, occasionally, in the cellular space of the arachnoid.

They believe that the cells filled with India ink reached the pia and arachnoid membrane after wandering from the blood vessel into the meninges. In view of the fact that normally India ink is deposited in the meninges by way of the phagocytic cells, they suggest the possibility of certain microorganisms reaching the meninges in the same way.

BLOOD SEDIMENTATION RATES IN MIDDLE AGED AND OLD PEOPLE*

ISIDORE MILLER, M.D., STATEN ISLAND, N. Y.

A PERUSAL of the literature reveals that little work has been done regarding the sedimentation rate in middle aged and old people. We were interested in ascertaining whether the sedimentation rate would be a simple method of diagnostic value to us in our examination of the aged, since our laboratory facilities are limited, x-rays and detailed laboratory examinations being done at a different institution.

This work was done at the New York City Farm Colony, a home for the aged, with a capacity of 1,300 beds, of which 250 are infirmaries beds. Six hundred and twenty-one sedimentation rates were taken on 496 apparently normal old men during the routine admission examinations. In this article, we are omitting the sedimentation rates obtained on our infirmaries cases.

Our method consisted in drawing 3 cc of blood from a cubital vein and emptying it into a test tube containing potassium oxalate as the anticoagulant. Blood was drawn into 1 cc pipettes, calibrated into hundredths, and the fall of the cells at the end of an hour was read. The pipettes were placed in racks lined with a thick rubber base, to which they were firmly pressed by elastic bands, running from the rack to the top of the pipettes. We considered a drop of 10 per cent the upper limit of normal.

This work was started two years ago. Of the 496 men examined, 127 have left the institution, 57 have died at the Farm Colony, and 312 are still present.

This study concerns itself with older men, especially those over fifty years of age. Degenerative diseases are in the ascendancy in this group, and the sedi-

*Received for publication November 6 1935

Remen found that 90 per cent of his 137 diabetic patients had an increased sedimentation rate. Kramer in a study of 366 diabetic patients found that 67.8 per cent showed abnormal readings. He also found that the blood sugar per se had no influence on the sedimentation rate. In our small group of 29 cases, 52 per cent showed an increase in the sedimentation rate.

Our tuberculous patients are mainly old fibrotic cases with negative sputa, and their sedimentation rates vary from normal to very high. One patient with a rate of 28 per cent advanced within six months to 45 per cent, at which time the sputum was Gaffky VI and the x-ray showed an advancement of the lesion. Our patients with syphilis, like those with tuberculosis, had rates varying from normal to very high. The tabetic patients had normal rates while those with central nervous system and cardiovascular involvement had increased rates.

SUMMARY

1. Sixty-six per cent of our patients had rates under 10 per cent, 86 per cent under 20 per cent.

2. Of our patients who died, 44 per cent had rates under 10 per cent, 72 per cent under 20 per cent.

3. Age has little effect on the sedimentation rate.

4. A single normal rate does not rule out pathology.

5. A high rate should be viewed with suspicion and should be cause for further study.

REFERENCES

- Cutler, J. W.: Practical Application of Blood Sedimentation Test in General Medicine, *Am. J. M. Sc.* 183: 643, 1932.
- Schattenberg, H. J.: Sedimentation Test as a Routine Laboratory Procedure, *Arch. Int. Med.* 50: 569, 1932.
- Remen, L.: Sedimentation Speed in Diabetes Mellitus, *Klin. Wehnschr.* 10: 2113, 1931.
- Kramer, D. W.: Blood Sedimentation Rate in Diabetes Mellitus, *J. LAB. & CLIN. MED.* 21: 37, 1935.

THE FREQUENCY AND SIGNIFICANCE OF CHANGES OF THE EXPIRATORY CHEST VOLUME DURING ROUTINE MEASUREMENT OF BASAL OXYGEN CONSUMPTION*

JAMES A. GREENE, M.D., IOWA CITY, IA.
WITH TECHNICAL ASSISTANCE OF MURIEL WARD

WHEN measured by the closed circuit method with the most careful technic, a basal metabolic rate is frequently obtained which is higher or lower than is consistent with the clinical condition of the patient. Errors in the basal metabolic rate produced by factors within the patients themselves have been considered extremely rare. The escape of gas through either a dilated lacrymo-nasal duct or a patent eustachian tube with a perforated eardrum accounts for most of these. Changes of the expiratory chest volume which occurred immediately before or during measurement of basal oxygen consumption were found by Greene and Coggeshall¹ to produce significant errors in the calculated results. The assumption that the same quantity of air remained in the lungs at the beginning and at the end of each measurement of oxygen consumption was recognized by Benedict² to be the weakest point in the closed circuit method. He was convinced after a large number of experiments that this factor was insignificant in normal subjects. According to Carpenter,³ changes in expiratory chest volume were insignificant in the earlier development of the method because the subjects were more or less trained, but became more significant when the subjects were unaccustomed to the apparatus. In fact, one of his subjects consistently added gas to the system instead of removing it.

Inasmuch as this source of error (changes in expiratory chest volume) has been ignored in the literature on clinical methods of measuring basal oxygen consumption, we have ascertained its frequency and significance in routine measurement of basal metabolic rate, and we have also noted the diseases in which the extreme changes may occur.

METHOD

Spirograms obtained during 2,985 routine measurements of basal oxygen consumption were examined for changes in the expiratory volume of the chest. The changes were manifested by alteration of the expiratory line of the spiogram and were classified into slight (1 cm. or less), moderate (1 to 2 cm.), and extreme (2 or more cm.).

We recognize that accurate measurement of changes in expiratory chest volume during measurement of basal oxygen consumption can be obtained only by simultaneous plethysmograms, but inasmuch as leaks in the system were ex-

*From the Department of Internal Medicine, State University of Iowa Medical School. Received for publication, November 18, 1935.

THE ACTION OF MAGNESIUM IN GUANIDINE INTOXICATION*

JOHN M. SAUNDERS, M.D., NASHVILLE, TENN.

INTRODUCTION

CALCIUM has been shown to protect the organism against the numerous pathologic manifestations produced by guanidine.¹⁻⁵ Fühner,⁶ working with an isolated nerve muscle preparation, showed that magnesium has a similar action to calcium in controlling muscle contractions caused by guanidine. This makes it interesting to know whether the many other manifestations of the intoxication seen when an intact animal is poisoned with guanidine can be controlled by magnesium. If this were true, the fact that large doses of magnesium can be given intramuscularly would make it a more convenient therapeutic agent than calcium to use in cases of clinical guanidine intoxication. Experiments were carried out to compare the effect of magnesium sulphate with that of calcium on the manifestations of guanidine intoxication.

COMPARISON OF PHYSIOLOGIC ACTION OF CALCIUM AND MAGNESIUM

The divalent ion magnesium may replace calcium in maintaining a suitable ionic equilibrium for certain biologic activities.⁷ However, on certain other physiologic functions magnesium and calcium have an antagonistic action.⁸ Although quantitatively different, the sedative effect of the two ions on the peripheral nervous system has been shown to be similar. It is well known that parathyroid tetany is due to a decrease in calcium and that the symptoms can be relieved by the administration of calcium.⁹ Several investigators¹⁰⁻¹² have demonstrated that magnesium is also effective in preventing and controlling the symptoms of parathyroid tetany.

The antagonistic action of calcium and magnesium is best illustrated by their effects on the central nervous system. Meltzer and Auer¹³ demonstrated the depressing effect of magnesium on the higher centers. They produced complete anesthesia in animals and showed that this anesthesia could be overcome immediately by the injection of calcium salts.⁵ In fatal magnesium poisoning, Meltzer and Auer¹³ demonstrated that respiration gradually becomes slow and shallow and stops before the heart action has ceased. This respiratory paralysis can be overcome and the function of the respiratory center completely restored by the administration of calcium. A further proof that calcium has a stimulating effect on the respiratory center is shown by the work of Hooker.¹⁴ He perfused the medulla of dogs with saline containing red blood cells and introduced solutions containing first an excess of calcium ions and then potassium ions. The perfusing solution containing an excess of calcium ions stimulated the respiratory center.

*From The Department of Pediatrics, Vanderbilt University Medical School.

Received for publication, October 3, 1935.

This work was aided by a grant from the Division of Medical Sciences of the Rockefeller Foundation.

The action of calcium and magnesium on carbohydrate metabolism is not well understood. Lang and Rigo¹⁵ produced a rise in blood sugar following the intravenous administration of moderate amounts of magnesium sulphate. Glycosuria following the intravenous injection of sea water was thought by Burnett¹⁶ to be due to the presence of magnesium in the solution. Underhill^{17, 18} used small doses of calcium and magnesium lactate subcutaneously and found that they failed to produce significant changes in the blood sugar content of rabbits, but the same amount of either salt intensified the effect of epinephrin on blood sugar. This suggests that the rise in blood sugar after the administration of small doses of calcium and magnesium is perhaps due to the adrenaal mechanism. Anesthetic doses of magnesium sulphate alone produce a marked increase in blood sugar.¹⁶ Stewart and Rogoff¹⁹ produced hyperglycemia following light ether anesthesia and asphyxia in adrenalectomized rabbits. This work suggests that the hyperglycemia produced by magnesium anesthesia is probably similar to the hyperglycemia produced by any other anesthetic and that the adrenaal mechanism has no part in it.

ACTION OF CALCIUM ON GUANIDINE INTOXICATION

Guanidine intoxication has been reported to occur clinically as a complication of various conditions^{3, 20, 24} and may be produced experimentally by the subcutaneous injection of guanidine hydrochloride. The symptoms of experimental guanidine intoxication are related chiefly to the nervous system and gastrointestinal tract. Nausea occurs early, followed by vomiting and diarrhea. There is hyperexcitability of the nervous system which is manifested by restlessness, incoordination, and tremors which increase until the subject has generalized convulsions. This hyperexcitability of the nervous system is, at times, followed by profound depression. Chemically, there are a hypoglycemia and an acidosis. The hypoglycemia has been shown to be largely due to an inability of the poisoned animal to metabolize lactic acid in a normal manner.^{2, 25} The lactic acid accumulates and leads to an upset in acid base equilibrium. However, a tendency has been noted for the pH to decrease out of proportion to the decrease in alkali reserve and this would indicate a depression of the respiratory center by guanidine.²⁶

Calcium has been repeatedly shown to protect the organism against the diverse manifestations of guanidine intoxication.^{2, 5} The lethal dose of guanidine is three or four times greater in animals kept on a high calcium diet than in subjects on an inadequate calcium intake.¹ The most efficient management of severe guanidine intoxication is to combine calcium therapy with other supportive measures, such as the forcing of fluids and the administration of glucose and an alkalinizing salt for the adjustment of the acid base balance.²⁶ Nevertheless, intensive calcium therapy alone gradually decreases or abolishes the manifestations. The vomiting and diarrhea subside. The activity of the nervous system gradually becomes more normal and respiration improves, indicating increased activity of the respiratory center.^{3, 4, 20, 27} There are an increase in the blood sugar and a decrease in lactic acid indicating a more normal metabolism.^{1, 2} These in turn tend to relieve the acidosis.

sents 26.67 per cent of total tests, ignoring the four cases of leucopenia without clinical symptoms and one case not brought to clinical tests, with full corroboration. This is a diagnostic value of the leucopenic index over skin tests of 21.91 per cent.

There were eleven foods clinically negative, but positive on skin test and without leucopenia, while four clinically negative foods, negative to skin tests, showed a leucopenia. One food productive of symptoms showed a positive skin test without the leucopenia. One other food was positive clinically with negative skin test and no leucopenia. This emphasized our earlier statement that the blood picture may not correspond with clinical symptoms and the leucopenic index is not to be accepted as absolutely specific, but its increased diagnostic

TABLE I

ANALYSIS OF 105 LEUCOPENIC INDEX COUNTS IN 23 CASES OF VASOMOTOR RHINITIS

CASE	DURATION YEARS	AL- LBERGENS TESTED	BLOOD COUNTS		SKIN TESTS		CLINICAL RESULTS		
			LEUCO- PENIA	LEUCO- CYTOSIS	POSITIVE	NEGATIVE	GOOD	FAIR	POOR
KKJ	1½	3	2	1	1	2			1
BT	7	9	4	5	1	8	1		
CO	3	7	5	2	1	6	1		
FJ	2½	4	1	3	2	2	1		
JJ	4	3	3		1	2		1	
TH	5½	3	1	2	2	1		1	
DLG	1½	3	1	2		3	1		
HE	12	2		2	1	1			1
MB	1½	3	1	2	1	2		1	
OB	½	3				3		1	
JB	50	10	3	7	6	4	1		
RG	9	4	3	1		4	1		
VK	6	8	2	6		8		1	
VN	4	6	2	4	1	5	1		
AP	3	4	1	3	1	3			1
CH	25	3	2	1		3	1		
MC	20	3	1	2		3	1		
JJ	4	7	5	2	3	4		1	
VV	5	3	2	1	1	2	1		
TLor	4½	4		4	2	2	1		
HH	6	4	2	2	1	3		1	
TLz	24	3	2	1	1	2	1		
EOB	1	6	3	3		6		1	
Totals		105	46	59	26	79	12	8	3

TABLE II

ANALYSIS OF 105 LEUCOPENIC INDEX COUNTS IN 23 CASES OF VASOMOTOR RHINITIS

	BLOOD COUNTS		CLINICAL TESTS			SKIN TESTS		PER- CENTAGE
	LEUCO- PENIA	LEUCOCY- TOSIS	POSITIVE	NEGATIVE	NOT TRIED	POSITIVE	NEGATIVE	
		45		45			45	42.86
		11		11		11		10.47
	4			4			4	3.81
		1	1			1		0.95
		1	1				1	0.95
	13		13			13		12.39
			28				28	26.67
	28	1			1	1		0.95
	1				1		1	0.95
Totals	46	59	43	60	2	26	79	
Totals	105		105			105		100.00

accuracy seems clearly demonstrated. Many cases of vasomotor rhinitis are refractory to allergic management, and because of negative skin tests, failure to respond to elimination diets, vaccines, glandular therapy, and other therapeutic procedures, are frequently classed as being nonallergic in character. It is this type of case in which the leucopenic index is particularly valuable. Rigid dietary regimes are often imposed on these individuals, selecting foods which are considered least likely to produce symptoms. And yet, the very foods which have been selected have often been demonstrated as contributory in the etiology of the condition. The leucopenic index will often establish such foods as lettuce, coffee, spinach, celery, rice, asparagus, apples, peaches, and other fruits and vegetables, as a cause of symptoms, and previously not given due consideration as such because of the limitations of the usual diagnostic procedures. Not only can a more specific diet be prescribed in a much shorter period of time, but the diet can often be planned to include some of the staple foods, such as wheat, eggs, milk, and potatoes, all or most of which are excluded from the usual trial diets imposing inconveniences and hardships to the patient.

With respect to inhalants, these findings would seem to apply as well to airborne allergens as to ingested agents. One patient, known to be sensitive to dog hair, complained of a "scratchy" feeling in the throat within ten minutes after exposure to this specific agent. This was followed by the development of itching and congestion of the nasal mucosa. Meanwhile the white blood count dropped 500 cells at the ten-minute period, and the leucocyte count fell to 1,400 cells below the fasting count within twenty minutes from the time of the initial exposure. At the end of an hour the leucocytes again approached the fasting count, the symptoms having subsided as the normal white blood cell count was regained.

Sensitization, even in the absence of clinical symptoms, cannot be ruled out when the allergen in question results in a markedly lowered white blood count. An increase in the test dosage may result in clinical symptoms which corroborate the blood findings. Degrees of sensitization may vary in the same patient from week to week and such changes are reflected in the blood picture as the sensitizations change. Differential blood counts were undertaken in a number of these leucopenic observations with the result that, at times, an increasing eosinophilia was seen to occur as the white blood count decreased, but the observation was so infrequent that its diagnostic import did not seem to warrant the procedure.

CONCLUSIONS

From the data in hand, it appears that the diagnostic accuracy of the leucopenic index materially exceeds the usual skin tests and clinical routines. It is of particular value when intractable conditions show few or no positive skin tests and fail to respond to usual allergic management. The superiority of the procedure over the usual diagnostic methods more than compensates for the time and painstaking effort involved. The leucopenic index facilitates more rapid and accurate diagnosis leading to improved specific therapeutic measures and results in vasomotor rhinitis.

REFERENCES

1. Vaughan, W. T.: Food Allergens. III. The Leucopenic Index, *J. Allergy* 5: 601, 1934.
2. Gay, L. P.: The Leucopenic Index as a Method of Specific Diagnosis of Allergens Causing Peptic Ulcer, *J. A. M. A.* 106: 969, 1936.
3. Rinkel, H. J.: The Leucopenic Index in Allergic Diseases. Read before American Association of Allergy, Atlantic City, June 10, 1935. Unpublished.
4. Vaughan, W. T.: Further Studies of the Leucopenic Index in Food Allergy, *J. Allergy* 6: 78, 1934.
5. Zeller, Michael: The Leucopenic Index in Intractable Asthma, *J. Ill. Med. Soc.* 1: 54, 1936.
6. Rinkel, H. J., and Gay, L. P.: The Leucopenic Index, *J. Missouri M. A.* 33: 182, 1936.

THE LEUCOPENIC INDEX AS A DIAGNOSTIC METHOD IN THE STUDY OF FOOD ALLERGY

WITH A DISCUSSION OF ITS RELIABILITY

WARREN T. VAUGHAN, M.D., RICHMOND, VA.

THE leucopenic index was developed in 1933 by the writer¹ as an outgrowth of an observation he had made some years previously, that although the Widal colloidoelastic reaction was not a reliable test for liver function, certain persons allergic to milk reacted with leucopenia after the ingestion of milk during the test. This suggested the possibility that it could be used for the demonstration of milk allergy rather than as a liver test. Also that, if the test should prove specific, it could be used with other foods to demonstrate the presence or absence of allergy.

This was found to be true in a sufficiently high proportion of cases to justify the development of a method for clinical use. The ingestion of a food to which a person is allergic will usually produce a leucopenic response, with a drop in total white count of more than 1,000 cells per c.mm. A food to which one is not allergic usually produces no such drop, may indeed be followed by a rise.

The technic of the leucopenic index is briefly as follows: Two fasting white counts are made at ten-minute intervals. The patient then eats the food for which he is to be tested. Following this, three additional counts are made at half-hour intervals. If any of the postprandial counts falls more than 1,000 below the mean of the two fasting counts the index is considered positive. A drop of less than 1,000 is considered negative unless it is maintained throughout the three counts, in which case it is described as a "general low range, probably positive." The same pipette and counting chamber are used throughout each study. Pipettes are shaken three minutes to ensure even distribution. Counting chambers are filled evenly and to approximately the same degree each time. At least 200 cells are counted (eight 1 mm. squares) in each determination. Blood is obtained from a freely flowing needle puncture.

Applying this method clinically we found in a series of 191 tests that 118 or 62 per cent showed a correspondence between the skin reaction and the

leucopenic index.² If the skin reaction was positive, the leucopenic was also positive and vice versa. In 21 the leucopenic index was positive although the skin reactions had been negative. Ten of this latter group found that the substances causing leucopenia also caused allergic symptoms. In these the index was the only means by which we were able to identify offending foods.

This first series represented patients with unknown sensitizations in which the index was used with the skin test, to determine etiologic factors. A second series³ was then run with patients whose idiosyncrasies were known prior to the application of the test. Twenty-six patients, known and proved to be allergic to stated foods, were tested both by the leucopenic index and the skin test. Twenty-one reacted positively to the offending food by the index, 17 by skin test. There was 81 per cent correspondence between food allergy and the leucocytic response as contrasted with 65 per cent with the results of skin testing.

Forty-three control tests with the same foods, in non-sensitive individuals gave but six positive leucopenic indices. One of these, a positive reaction to tomato, occurred in an individual who had undoubtedly previously been sensitive to tomato although he had ceased some time previously to experience symptoms therefrom. The same may have been true in some of the other false positive reactions.

In this second series the test was carried out for a total period of four hours. The most pronounced drop sometimes did not occur until three and one-half or four hours after ingestion. For the simplification of the test, it was found that if it be run for but an hour and a half, about 8 per cent of positive reactions would be missed. In using the index one should therefore, bear in mind that there may be delayed positives which do not show up within the arbitrary test period. Therefore, in case of doubt this period should be prolonged to two, three, or even four hours.

It was shown that the leucocytic response to wheat, egg, and milk was at a lower level in allergies than in nonallergies. That is, there was as much fluctuation up and down from highest to lowest counts, but the total range for allergies occurred in a lower band of total white counts than the nonallergies. This is probably equally true for other foods. The general upward trend of the non-sensitive response tends to confirm the conception of a low grade normal digestive leucocytosis in some persons.

The usefulness of the leucopenic index has been confirmed by Rinkel⁴ who has brought out the fact that intractable asthmatics sometimes give positive reactions to nearly all foods, by Zeller⁵ who emphasizes its value in intractable asthma, by Gray⁶ who recommends its use in the study of peptic ulcer, and by Squier⁷ who suggests that additional information can be obtained if total eosinophile counts are done synchronously with total white counts.

The present communication deals particularly with an intensive investigation of a single patient who is so unfortunate as to be allergic to a very large number of foods and who, fortunately for the study, responds symptomatically very promptly and in a clear-cut manner to offending foods.

Her symptoms include migraine, urticaria, angioneurotic edema and colonic pain.

She was tested by the standard technic with 31 foods. Sixteen failed to cause symptoms. With one exception none of these foods was followed by a leucocyte drop of 1,000 or more (Chart 1).

Fifteen foods caused symptoms. Every one of these was followed by a drop of more than 1,000 at some point in the postprandial curve (Chart 2). The severity of resultant symptoms is indicated on the chart in degrees of 1-plus, 2-plus, etc. With one exception (grape) the severity of the reaction corresponded quite closely to the degree of depression of the white count.

Charts 3, 4, and 5 show the total range of the curves in response to foods causing no symptoms, mild symptoms and severe symptoms. The more pronounced depression, accompanying more severe reactions, is evident. The response to honeydew in Chart 4 is of especial interest. At the end of ninety

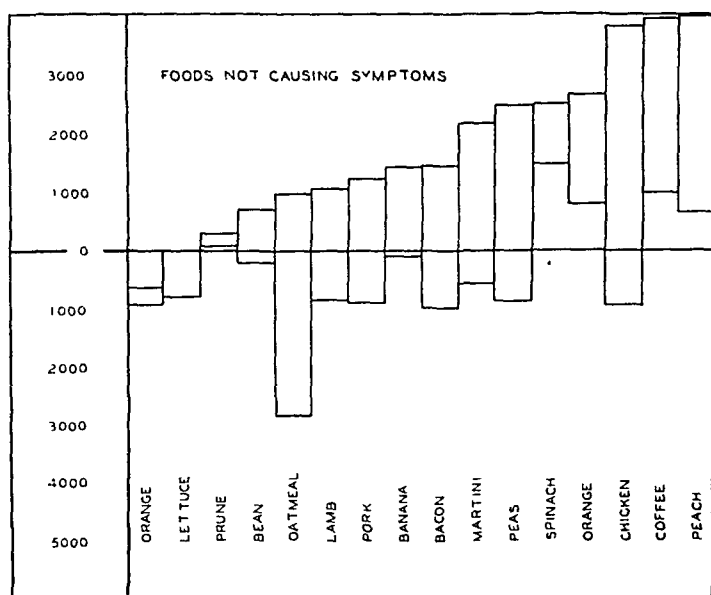


Chart 1.

minutes (the standard test time) the index was still negative but the patient had already responded with migraine. An additional count was therefore done at the end of two and one-half hours, at which time the index had become positive. This was a delayed positive reaction, a false negative index with the test as routinely applied. It emphasizes the need for continuing the count in case of doubt.

The curves of response to pork are of interest (Chart 6). Obviously, ham, bacon, pork, and pork sausage are entirely distinct foods, but, irrespective of other ingredients and methods of preparation, they all do contain pork. With the exception of ham there is a general similarity to the curves, all of them being of the "Z" type, suggesting the possibility of a consistent curve of response to a given food.

The most interesting feature of the pork curve is the observation that pork at one time produced a positive index, *with accompanying symptoms*, while

at another time the index was negative and *there were no symptoms*. We may speak of the patient in the latter case as being sensitive to pork but in allergic equilibrium at the time of the test. These two types of response to pork in

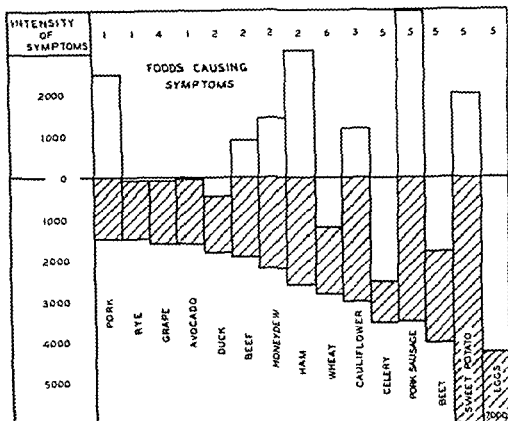


Chart 2.

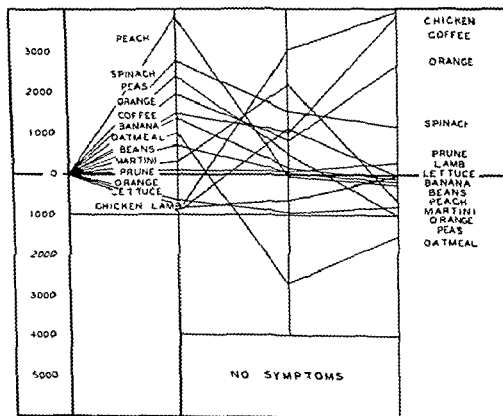


Chart 3

the same patient justify the designation of an 800 drop or thereabouts as "borderline" and as indicating that the patient may actually be allergic to this food. In this event repetition of the test would be in order.

DISCUSSION

If the leucopenic index is to establish itself as a diagnostic measure useful in food allergy, it must fulfill certain criteria. It must be based upon estab-

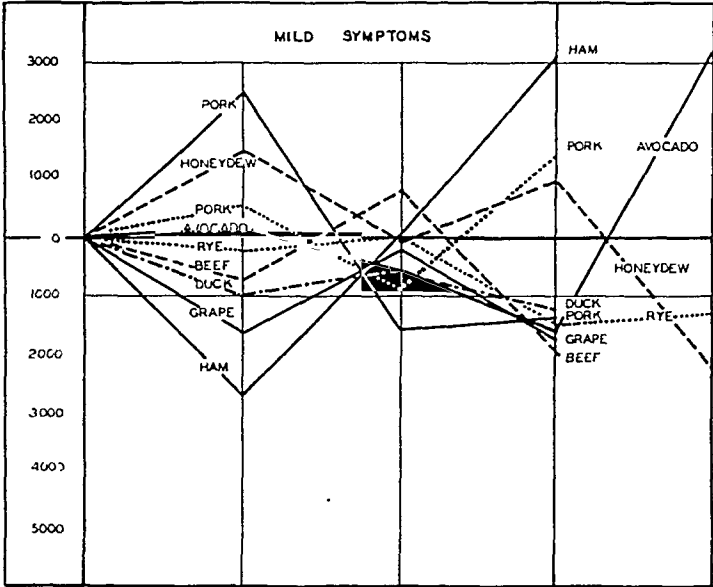


Chart 4.

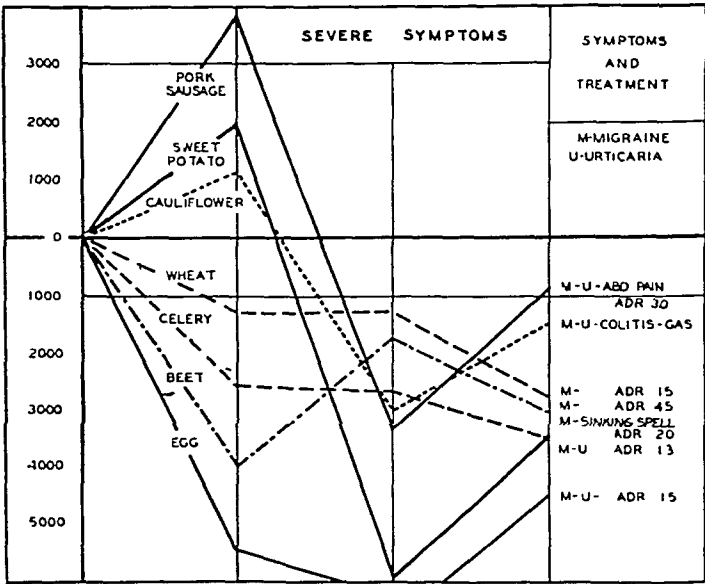


Chart 5.

lished facts. It must be the measure of a response which is the rule rather than the exception. It must have a reasonably high degree of accuracy. It must be made as simple as possible consistent with reasonable accuracy.

Of the occurrence of digestive leucopenia following the ingestion of an allergenic food there can be no doubt. Leucopenia forms part of the picture of experimental anaphylactic shock. My own observations and those of the authors above mentioned demonstrate a clinical correlation. I have recently been fortunate in obtaining Joltiam's monograph⁸ on the "Hemoclastic Crisis in Urticaria" which contains numerous, sometimes startling illustrations of leucopenic response to the ingestion of a specific allergen. In it Joltiam states that Vidal's original work on the hemoclastic or colloidoclastic crisis dealt with it as an allergenic response. It was not until later that he suggested its use as a test for liver function.

The question of the most desirable procedure for the routine test may still be an open one. In our clinic we are using the routine and criteria which

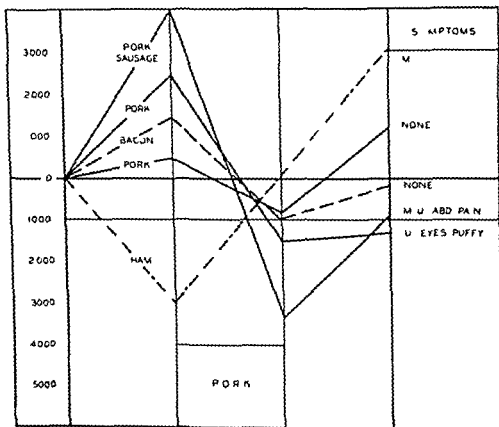


Chart 6

I first suggested in 1934, except that instead of taking the mean of the two fasting counts as a base line, we now take whichever of the two counts is the lower and require a drop of 1,000 cells or more below this lowest fasting count.

A drop of 1,000 is an arbitrary requirement but is based upon the sound fact that in the vast majority of instances the fluctuation of total white counts in a fasting individual is usually not over 2,000. The upward fluctuation of 1,000 is ignored, the downward fluctuation of 1,000 is adopted as a standard. Occasionally, one sees much wider fasting fluctuations. In one case we observed a fluctuation, within ten minutes, of over 3,000. A number of factors enter into this, some of which are not controllable. The total number of leucocytes in one capillary bed may be quite different from that in an adjoining bed at the same instant. However it has been shown that simultaneous counts taken from different fingers are the same, within the limits of normal error. Accuracy of laboratory technique including method of puncture, amount of squeezing,

care in filling the pipette, consistent delivery of the same volume of fluid into the counting chamber, and accuracy in the actual counting are all factors that can be controlled up to a certain limit. An even distribution is *most important* and requires hand shaking of the pipette for at least three minutes. Extreme accuracy throughout the procedure is indispensable.

Rinkel, likewise Gay, find that a drop of but 500 cells may be significant. Since this fluctuation may be exceeded in the fasting state and since it is scarcely outside the limits of error in white counting, I feel that a 1,000 drop is a safer criterion. Joltrain believes that a drop of 2,000 cells or more is indicative of a shock reaction. He, however, has dealt with explosive cases with pronounced allergic symptoms, reactions that correspond more to those illustrated in Chart 5, above. A mild but definitely positive reaction which we so often observe would be overlooked if we adopted the criteria of Joltrain. For the present therefore I feel that the original standards of interpretation should be retained, except as mentioned, regarding the fasting level. A drop of between 500 and 1,000 may be designated "suggestive" or "borderline"; one of 1,000-2,000 as positive; over 2,000 as strongly positive.

Is the test sufficiently reliable to be utilized effectively? The answer to this question involves a recapitulation of interpretive factors. Too wide a fasting fluctuation may obscure the findings. I have gained the impression that the higher the fasting white count the more likely is there to be a wider fluctuation, although this does not always occur. Also, the higher the white count the more pronounced will be the drop in a positive response. This raises a question as to whether the requisite drop should vary depending upon the original fasting count. Thus, a drop of 1,000 might be required if the fasting count is 6,000 while a drop of 2,000 would be required if the fasting count is 12,000 or 14,000. On this I have as yet no final opinion.

We have seen patients who have reacted positively one day, and who on the next morning still showed leucopenia (around 3,000). We have interpreted these as still showing a delayed positive index. Since the patient is still reacting, one would not expect a further drop, following the ingestion of another allergenic food. This might also occur following the eating of an allergenic food, even though no test was being done. A fasting leucopenia therefore decreases the reliability of the test.

The delayed positive reaction will be missed with the routine procedure. The examiner should be conscious of this fact and be ready to prolong or repeat the test in case of doubt and especially if the final count suggests a beginning drop. Since 92 per cent of positive indices will be so within ninety minutes this justifies simplification, but at the same time completion of the test at ninety minutes diminishes the accuracy of the findings.

Foods which actually cause trouble may at times give borderline and negative indices. This is illustrated in the pork chart (Chart 6). One should realize this and repeat the test where indicated. It is of especial interest to note that in the case described, when the test was negative the patient did not respond with symptoms.

The test may occasionally be positive to a substance which does not cause trouble. In one case (tomato) this was interpreted as a residual effect of a known previous sensitization.

We have run repeated tests with the same food in quite a number of individuals. In roughly 50 per cent the index remained consistently the same, either positive or negative, but in 50 per cent there was a change from positive to negative or vice versa. At first I thought that this indicated an inherent weakness in the test but after further study I have concluded that the factor of variation was more likely to be in the individual, as in the pork test, particularly in view of the fact that these repetitions were done on only very mildly sensitive individuals. It is well known that persons mildly sensitive to certain foods often may eat these foods with impunity just as frequently as they eat them with consequent symptoms. Very highly sensitive persons with pronounced drops tend more to give similar responses each time.

The leucopenic index is not proposed as a substitute for other methods of allergic study. No one procedure can be counted upon to solve the intricate problems of the objective diagnosis of food allergy. The index, with an accuracy of 81 per cent when used as a routine procedure or 93 per cent when performed with utmost care as in the case herein detailed, compares very favorably with the skin test to foods, with its reliability of 65 per cent. However, it possesses the disadvantage that it is most time consuming. We have found it to be a very helpful adjunct to the skin test, using it routinely with a few staple foods, and with other selected foods concerning which there may still be special question.

ADDENDUM

The reliability of the leucopenic index has recently been questioned⁹ on the basis of published reports on the great variability of the fasting leucocyte count. The reports of eight investigators or groups of investigators are marshalled to demonstrate that there is such a wide normal variation in total leucocyte counts that conclusions drawn therefrom are open to serious doubt. An analysis of these eight studies is as follows:

It is quite true that there are wide fluctuations in the leucocyte count, both fasting and postprandial, but these occur in the afternoon rather than in the morning. Leucopenic index studies are done in the morning, the patient reporting to the office in the fasting state at 9 o'clock. The study requires about two hours and we usually make only one per day on each patient.

Sabin and her collaborators¹⁰ report all day studies on six persons. Their total ranges in white count from 9 to 11 AM in these six series are as follows: 2,000, 1,700, 1,800, 2,500, 2,500, 3,700. Taking the interval 9 to 12 AM we find a wider variation as follows: 2,500, 3,000, 3,000, 3,000, 3,500, 6,000. The range through morning and afternoon is much wider, as follows: 6,000, 4,700, 5,200, 6,000, 4,300, 5,000.

Medlar¹¹ has observed a variation of 50 per cent in less than half an hour. But in his serial counts which were made from 9 to 10 AM in two cases and 9 to 11 in one, only six out of a total of 59 counts showed a fluctuation of more than 2,000. Furthermore, since he was making his counts at three and five

accuracy with this simplification. I have found that we lose about 8 per cent in accuracy. The index, as used routinely, is therefore not 100 per cent accurate or reliable but even so it is about 20 per cent more reliable than the skin test to foods (scratch, intracutaneous, or both).

I feel justified in concluding that the bulk of the evidence reviewed warrants the statement that our interpretation of the leucopenic index, as it is done, in the morning hours, is consistent with the available literature on variations of the normal white count.

REFERENCES

The author wishes to express his appreciation of the interest and collaboration of Mr. and Mrs. Ray A. Van Clief of Esmont, Virginia, who have made possible the study of the leucopenic index as it has been developed to date.

1. Vaughan, Warren, T.: Food Allergens. III. The Leucopenic Index. Preliminary Report, *J. Allergy* 5: 601, 1934.
2. Vaughan, Warren T.: Further Studies on the Leucopenic Index in Food Allergy, *J. Allergy* 6: 78, 1934.
3. Vaughan, Warren T.: Food Idiosyncrasy as a Factor in the Digestive Leucocyte Response, *J. Allergy* 5: 421, 1935.
4. Rinkel, Herbert J.: The Leucopenic Index in Allergic Diseases, *J. Allergy* 7: 356, 1936.
5. Rinkel, Herbert J.: The Leucopenic Index. II. Concerning the Nature of Food Sensitization in Intractable Allergic Diseases, *J. LAB. & CLIN. MED.* 21: 814, 1936.
6. Zeller, Michael, The Leucopenic Index in Intractable Asthma, *Illinois M. J.* 69: 54, 1936.
7. Gay, L. P.: The Leukopenic Index as a Method of Diagnosis of Allergens Causing Peptic Ulcer, *J. A. M. A.* 106: 969, 1936.
8. Squier, Theodore E.: *Am. J. M. Sc.* In Press.
9. Joltrain, Edouard, Les Urticaires: Crises Hemoclasiques, Doin, Paris, 1930.
10. The Leukopenic Index and Food Allergy, *J. A. M. A.* 106: 1988, 1936.
11. Sabin, Florence R.; Cunningham, R. S.; Doan, C. A.; and Kindwall, J. A.: The Normal Rhythm of the White Blood Cells, *Bull. Johns Hopkins Hosp.* 37: 14, 1925.
12. Medlar, E. M.: The Extent of the Variations in the Leukocytes of Normal Individuals, *Am. J. M. Sc.* 117: 72, 1929.
13. Jones, Edgar; Stephens, D. J.; Todd, Harriett; and Lawrence, J. S.: Studies in the Normal Human White Blood Cell Picture: I. Variations in Recumbent Basal Subjects and in Individuals With Change of Posture, *Am. J. Physiol.* 105: 547, 1933.
14. Shaw, A. F. B.: The Diurnal Tides of the Leukocytes of Man, *J. Path. & Bact.* 30: 1, 1927.
15. Mauriac, P., and Cabouat, P.: Contribution à l'étude des variations de la formule leucocytaire chez l'homme normal, *Paris méd.* 39: 407, 1921.
16. Ponder, Eric; Saslow, George; and Schweizer, Malvina: On Variations in the White Cell Count of Man, *Quart. J. Exper. Physiol.* 21: 21, 1931.
17. Simpson, R. H.: Physiological Leukocyte Counts and the Detection of Small Deviations from the Normal, *Brit. J. Radiol.* 6: 705, 1933.
18. Washburn, A. H.: Blood Cells in Healthy Young Infants: III. A Study of 608 Differential Leukocyte Counts, With a Final Report of 908 Total Leukocyte Counts, *Am. J. Dis. Child.* 50: 413, 1935.

LABORATORY METHODS

THE LABORATORY DIAGNOSIS OF BLASTOMYCOSIS*

DONALD S. MARTIN, M.D., AND DAVID T. SMITH, M.D., DURHAM, N. C.

RECENT reports in the literature prove conclusively that blastomycosis (American type) is a disease which is far more common and more widespread than is generally realized. The clinical picture of generalized infection with *Blastomyces dermatitidis* is frequently so similar to that presented by tuberculosis that the clinician is often compelled to turn to the laboratory to establish a diagnosis. Many of the cases reported as blastomycosis have been diagnosed solely upon the finding of the fungus in sections of organs or in sputum, pus, or skin scrapings from the infected patient. Isolation of the fungus from such material is frequently unsuccessful. Benham¹ has called attention to the fact that there are at least 10 fungi capable of causing "systemic infections with granulomatous lesions and which appear in the infected tissue as rounded cells more or less resembling yeasts." Although the fungus causing Gilchrist's disease has certain definite morphologic characteristics in lesions which are practically diagnostic in the hands of one experienced in their identification, it is obvious that confirmation by culture should be obtained when ever possible.

The isolation and identification of this fungus, a relatively simple procedure for the mycologist, frequently present some difficulties to the bacteriologist who is not accustomed to working with fungi and who does not have access at all times to the special media required for mycologic study.

We have succeeded in culturing the causative organism from 9 cases of human blastomycosis and from a dog with spontaneous pulmonary blastomycosis. The only media found necessary for the isolation of the fungus were blood agar plates pH 7.4 and Sabouraud's dextrose agar slants.

The bacteriologic procedures which are of definite value in establishing the diagnosis of infection with *Blastomyces dermatitidis* are

- 1 Direct microscopic examination of the unstained sputum, exudate, or scrapings
- 2 Isolation and identification of the infecting organism
- 3 Skin tests with killed organisms
- 4 Complement fixation tests

PROCEDURES

Direct Microscopic Examination—The material is first examined in the usual manner by mixing some of the sputum, pus, or scrapings with 10 to 20

*From the Departments of Bacteriology and Medicine, Duke University Medical School. Received for publication January 15, 1936.

Presented before the Society of American Bacteriologists, December 28, 1935.

per cent fresh sodium hydroxide or potassium hydroxide. The organisms are seen as double contoured round or oval budding forms usually 8 to 10 μ in diameter although they may be much larger. Frequently there may be difficulty in differentiating certain artefacts from true blastomycetes. In these cases it is often advisable to make fresh wet preparations without sodium hydroxide, rim the coverslip with vaseline and allow the slide to set at room temperature for twenty-four to forty-eight hours. Blastomycetes in pus and sputum

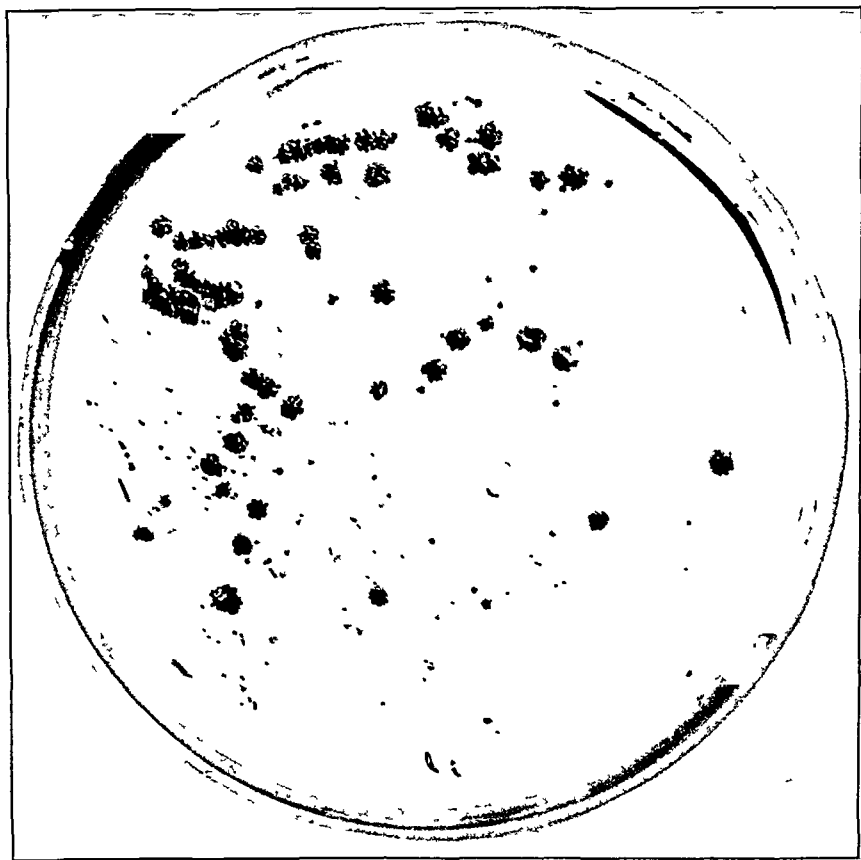


FIG. 1 —Photograph of colonies of *Blastomyces dermatitidis* on blood agar incubated at 37°C for two weeks

under these conditions frequently germinate to form well-developed branched mycelia.

Isolation of the Fungus—Cultures of the fungus from the early cases were obtained by planting the infected material on Sabouraud's dextrose agar. Certain difficulties were encountered which led us to the adoption of the following method for routine isolation of *Blastomyces dermatitidis*. The specimens are planted by heavy inoculation on two or more blood agar plates and on several Sabouraud's dextrose agar slants. The slants and some of the blood agar plates are left at room temperature. The remaining plates are incubated at 37° C. All plates and slants are kept at least four weeks before discarding, since it

occasionally takes this long for colonies to develop. Colonies are usually apparent from one to two weeks after inoculation. Media which appear hopelessly contaminated with bacteria or yeastlike organisms should not be discarded, for the blastomycetes are often capable of growing and forming recognizable colonies in spite of the numerous other organisms present.

On blood agar incubated at 37° C, the colonies are small and waxy and do not differ greatly in appearance from colonies of *tubercle bacilli* (Fig 1). Aerial hyphae do not appear unless the cultures become very old or dry. The

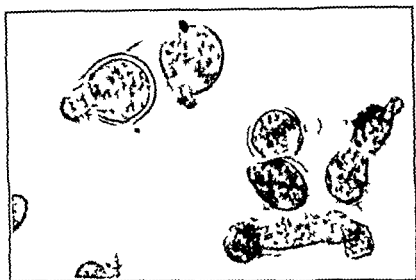


Fig 2—Photograph showing yeastlike cells obtained from one of the colonies shown in Fig 1. A drop of tincture of iodine was added to the fresh preparation.

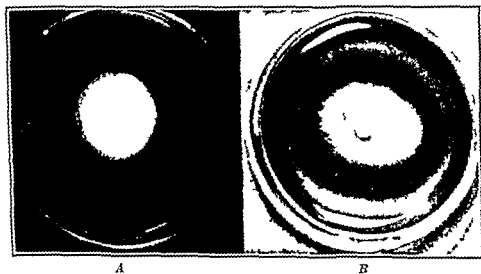


Fig 3—A Photograph of a colony of *Blastomyces dermatitidis* on Sabouraud's dextrose agar incubated at room temperature for eight days. B Photograph of a colony of *Blastomyces dermatitidis* on blood agar incubated at room temperature for eight days.

colonies are easy to pick up with an inoculating loop as there are no mycelia holding them to the medium. Smears of this material show numerous budding yeastlike structures with occasional elongated forms resembling rudimentary mycelial elements (Fig 2).

On Sabouraud's dextrose slants the organism appears first as a white mold with an abundant aerial mycelium (Fig 3 a). Microscopic examination shows numerous branched hyphae with lateral conidia and thick swollen structures resembling chlamydospores. Raquette hyphae such as are found in cultures of dermatophytes may be seen (Fig 4, a).

On blood agar at room temperature, the fungus forms a large colony which is characterized by the formation of a mycelium in the medium. The surface growth is small and slightly elevated. Radiating in an orderly manner from this central "button" are the densely packed mycelial strands (Fig. 3, *b*). The colony is so firmly appressed to the medium that it cannot be picked up easily with a bacteriologic loop. On microscopic examination, the colony is seen to be made up chiefly of densely packed intertwined and curled hyphae. Conidia are rare, but some double contoured bodies as well as many large swollen thick-walled chlamydospores are present (Fig. 4, *b*). If the culture is allowed to develop further, an aerial mycelium is formed which may either be soft and fluffy in appearance or the projecting hyphae may grow as if twisted together in coarse coremiform structures.

The yeastlike form of the organism can be maintained by subculturing the organisms on blood agar. After good growth has occurred, the slants are



Fig. 4.—A, Photograph showing the microscopic appearance of a fresh lactophenol preparation obtained from the colony seen in Fig. 3 A stained with 0.5 per cent cotton blue. B, Photograph showing the microscopic appearance of a fresh preparation from the colony seen in Fig. 3 B stained with tincture of iodine.

placed in the ice box until the time of next transplant. If the cultures are removed from the incubator and left at room temperature, aerial hyphae appear within several days. One strain has been maintained in the yeastlike form for four and one-half years. Cultures kept in the moldlike stage by repeated transplants on Sabouraud's medium will develop into waxy yeastlike colonies if subcultured on the blood agar medium. Typical colonies on blood agar were obtained in this way from two strains which had been kept moldlike for fourteen months.

Skin Tests.—We have used a heat-killed suspension of blastomycetes as skin testing material. The vaccine is prepared by suspending some of the yeastlike organisms from the waxy growth on blood agar in sterile saline. The vaccine is diluted until the resulting suspension is faintly cloudy and is then heated to 60° C. for four hours. A sample of vaccine is planted on blood

agar and incubated at 37° C for at least ten days to insure sterility. The vaccine is preserved with 0.35 per cent thimerosal. Skin tests are done by injecting 0.1 cc of this suspension intracutaneously. Usually an area of erythema appears about the site of injection within fifteen to twenty minutes. This reaction, however, is not specific, since we have found it in patients with other types of pulmonary infection. The characteristic reaction begins after twelve to twenty-four hours and reaches its maximum in two or four days, resembling very closely a positive tuberculin reaction. Occasionally the reaction progresses to sterile abscess formation.

Complement Fixation Tests—A complement fixation test specific for *Blastomyces dermatitidis* has recently been described by one of us.² The antigen for the test is prepared from the fungus grown for two weeks on blood agar at 37° C. The organisms from 2 or 3 slants are washed off in 3 or 4 cc of normal saline and ground in a mortar to break up the larger clumps. The anticomplementary titer of the suspension is determined as follows. Normal saline is added in such a way as to obtain dilutions of the original suspension representing 100 per cent, 90 per cent, 80 per cent, etc., to 10 per cent. The quantity of each suspension to be tested should be determined by the customary quantities used in the routine Wassermann test in the laboratory where the test is to be performed. In this laboratory the procedure is to add 0.2 cc quantities of each dilution to 0.2 cc of saline and 0.2 cc complement. This mixture is placed in the ice box for three hours. The rack is then warmed for thirty minutes in the 37° C water bath. Sensitized cells (0.4 cc) are added, and after thirty minutes' incubation the degree of hemolysis in each tube is determined.

The suspension used in the actual test is one third of the greatest dilution which shows the least anticomplementary properties. The patient's serum after inactivation at 56° C for twenty minutes is diluted 1:4 with saline and 0.2 cc quantities added to the same amounts of complement and properly diluted antigen. Fixation is carried out at ice box temperature for three hours, and the rest of the procedure is the same as used in determining the AC titer of the antigen. The results of the test are recorded as 1+, 2+, 3+, and 4+ depending upon the degree of inhibition of hemolysis. The serum and antigen should be controlled for anticomplementary properties by using double quantities (0.4 cc) of each reagent.

Minor difficulties and variations in the laboratory diagnosis in individual cases are best illustrated by reporting our experiences with the following patients.

CASE 1—M. M. was admitted to Duke Hospital with a diagnosis of metastatic carcinoma of the lung. Direct examination of the sputum showed occasional yeastlike bodies, some of which were double contoured. On Sabouraud's medium a moderately heavy growth of monilia appeared several days after inoculation. However, after two weeks' incubation, typical colonies of *Blastomyces dermatitidis* appeared and the organisms were obtained in pure culture by the usual plating methods. No skin tests or complement fixation tests were done. The patient died and at necropsy the blastomycetes were found by direct examination of lung tissue.

CASE 2—W. G. Bloody pus was obtained from fluctuating abscesses on the hands and feet. No double contoured organisms were seen on direct examination, but pure cultures of

the fungus were obtained on both Sabouraud's dextrose agar and blood agar. Subculture to Sabouraud's medium failed to grow until laked blood was added to the medium. Subsequent transplants were successful without the addition of blood. Colonies on the original blood agar plate were subcultured on blood agar slants and have maintained their waxy growth and yeastlike form on this medium for a period of four and one half years. Transplants were made every two weeks during the first year, and the interval between transplants has been gradually lengthened until now they are subcultured every two or three months.

No skin tests or serologic studies were done. The necropsy findings in this case were those of extreme generalized blastomycosis involving practically every organ in the body. Blastomycetes were seen in sections of all lesions.

CASE 3.—R. B. This patient was admitted to a sanatorium with a diagnosis of pulmonary tuberculosis complicated by a pleural effusion. The sputum was negative for tubercle bacilli by direct examination and guinea pig inoculation. Specimens of sputum were planted on Sabouraud's medium and incubated at 37° C. A heavy growth of monilia appeared four days after inoculation and the typical moldlike growth of blastomyces appeared twelve days after inoculation. No skin tests or complement fixation tests were done.

CASE 4.—S. D. Pure cultures of the fungus were easily obtained by planting pus from the chest wall on blood agar and Sabouraud's medium. Sputum cultures revealed recognizable colonies which appeared only after two weeks' incubation. Skin tests were positive. Repeated serologic tests over a period of seven months showed uniformly positive fixation of complement with his own organism as antigen as well as with antigens prepared from 2 strains of blastomyces isolated from other patients. The patient died and extensive blastomycosis was found at necropsy.

CASE 5.—E. L. The first specimens of sputum were overgrown by aspergilli. Blastomycetes, however, were recovered in pure culture on blood agar and Sabouraud's media inoculated with pus from a subcutaneous abscess on the thigh. Treatment with potassium iodide was started and after several weeks the aspergilli disappeared suddenly from the sputum and blastomycetes were recovered on both types of media. Skin tests were positive as were the complement fixation tests with two different strains of blastomyces as antigen. The patient died but necropsy was not permitted.

CASE 6.—G. J. This was a case of extensive skin blastomycosis in which numerous budding forms were seen on section and on direct examination of scrapings from the skin. On first admission to the hospital, scrapings were planted on Sabouraud's medium and blood agar but growth was not obtained since the plates were discarded because of the extreme degree of bacterial contamination. On the second admission positive cultures were obtained only on the blood agar plates which were allowed to set at room temperature for three weeks. Because of the very heavy growth of bacteria, pure cultures could be obtained only by clipping the most superficial hyphae with sterile finger nail scissors and transplanting the clippings to fresh media. Skin tests were negative. Complement fixation tests with one sample of serum were negative with 5 strains of blastomyces. The fungus recovered from this patient fixed complement when mixed with the serum from Case 8.

CASE 7.—A. Y. This patient was admitted with six large fluctuating subcutaneous abscesses. Double contoured organisms were seen on direct examination of pus from one of these abscesses. Because of certain points in the history, one of the attending physicians suspected that this was a case of infection with cryptococci and not Gilchrist's disease. A fresh preparation allowed to set overnight revealed that the organisms produced definite branching mycelia in the pus. This observation ruled out the possibility of cryptococcus infection (Torulosis). Pus planted on the routine media yielded pure cultures on all media. Skin tests were markedly positive and resulted in the formation of a small sterile abscess at the site of injection. Repeated complement fixation tests were negative with 5 strains of blastomyces, including the strain isolated from the patient. The blastomycete recovered from this patient fixed complement with serums from Cases 8 and 9.

CASE 8—V S This patient was admitted with the clinical diagnosis of primary carcinoma of the lungs. A specimen of material obtained at bronchoscopy showed typical blastomycetes by direct examination and growth was obtained on all the routine media. This patient later had a severe blastomycotic infection of the uterus and peritoneum. Cultures of the pelvic peritoneal organs as well as the peritoneal pus were positive on all routine media. It is interesting to note that typical colonies appeared more rapidly on the blood agar plates kept at room temperature. A skin test was markedly positive with subsequent formation of a small sterile abscess. Repeated complement fixation tests over a period of fifteen months have been strongly positive with seven strains of blastomycetes including the strain isolated from the patient's sputum.

CASE 9—R H Inoculations of sputum, urine and pus from subcutaneous abscesses by the routine method yielded blastomycetes in all instances. Skin tests were questionable. Complement fixation tests were positive with 6 strains of blastomycetes. Necropsy of this patient showed blastomycotic lesions in the lungs, subcutaneous tissues and prostate.

CASE 10—Dog with spontaneous pulmonary blastomycosis. Smears of the lung made at postmortem showed typical double contoured budding forms. Cultures of the lung on Sabouraud's medium resulted in numerous colonies of monilia resembling those described by Benham³ as *Monilia krusei*. After several weeks aerial hyphae appeared and pure cultures were finally obtained by the clipping method described under Case 5. No serologic studies were made of this organism.

SUMMARY AND CONCLUSIONS

The laboratory procedures of value in the diagnosis of blastomycosis are discussed and may be summarized as follows:

The usual method of direct examination of sputum or pus may be supplemented by keeping a fresh specimen of the material at room temperature for twenty-four to forty-eight hours. The formation of a branched mycelium proves that a suspected structure is a fungus.

Sabouraud's dextrose agar is valuable as an isolation medium, but cultures should be planted also on blood agar since some strains apparently require blood in the medium for primary isolation.

Specimens apparently hopelessly contaminated with yeasts or bacteria frequently will yield colonies of blastomycetes if the cultures are kept for three to four weeks.

Pure cultures of the fungus often can be freed of contaminating organisms by clipping the aerial hyphae with sterile finger nail scissors and transplanting the clippings to suitable media.

Descriptions and photographs of the macroscopic and microscopic appearances of the fungus when grown on blood agar and Sabouraud's medium are presented.

The yeastlike form of the organism can be maintained on blood agar for as long as four and one-half years and frequently can be recovered from the moldlike type of growth when subcultured on blood agar. This reversion has been accomplished in two strains which were maintained in the moldlike stage for fourteen months.

A positive tuberculin-like skin test with a heat-killed vaccine prepared from the yeastlike form of the organism is obtained in some cases of blastomycosis.

Complement fixing antibodies are present in the serums of a large percentage of patients with generalized blastomycosis.

It has been demonstrated by the complement-fixation reaction that seven strains of blastomyces from seven cases of Gilchrist's disease are closely related antigenically and that any one of these strains may be used as antigen in the test. This suggests that a stock strain of *Blastomyces dermatitidis* may be kept in the laboratory and used as a source of material for skin tests and complement-fixation tests. This should be of value in early diagnosis since isolation and identification of the infecting organism may require several weeks.

All the procedures described above can be carried out with materials and media present in most routine bacteriologic laboratories.

REFERENCES

1. Benham, Rhoda, W.: The Fungi of Blastomycosis and Coccidioidal Granuloma, Arch. Dermat. & Syph. 30: 385, 1934.
2. Martin, Donald S.: Complement-Fixation in Blastomycosis, J. Infect. Dis. 57: 291, 1935.
3. Benham, Rhoda, W.: Certain Monilias Parasitic on Man, J. Infect. Dis. 49: 183, 1931.

SCHILLING'S HEMOGRAM*

EMIL MARO SCHLEICHER, A.B., DETROIT, MICH.

THE differentiation and enumeration of the cellular elements in a blood smear have become a highly technical aspect of clinical hematology since the publication of Arneth's "Neutrophilic Index"¹ (shift to left and right) and Schilling's "Hemogram" methods.²

From a study of articles pro and con in the current laboratory and clinical journals, it appears that Schilling's hemogram is favored by the majority of writers. But in spite of the fact that the hemogram has been found to be a valuable aid in clinical hematology, it seems that the method is only beyond criticism if modified or combined with an auxiliary method. It is not within the scope of this paper to discuss the various modifications suggested. It is agreed that Arneth's "neutrophilic index" is cumbersome, and that no one laboratory method should be considered absolute. However, it is my contention that the modifications of the hemogram method as presented in the current literature are not necessary if a blood smear is studied in a systematic manner.

Schilling's hemogram method has been used in this laboratory for the past five years, and in no case was it necessary to modify the method in order to detect conspicuous deviations of the differential from the normal. The reason for this lies chiefly in the early realization that only a *systematic* study of a blood smear would at all times insure a successful application of the hemogram. The method about to be described has as yet not failed to reveal what is termed by Schilling† "die biologische Reaktion einer spezifischen Krankheit."

For those who are not well acquainted with the numerical relationships of the neutrophilic elements of Arneth's and Schilling's indexes, Fig. 1 illustrates what is discussed in detail elsewhere.³ The maturation process of the myeloid cell and its numerical appearance as a neutrophile in normal blood is demon-

*From the Anemia Laboratory, Harper Hospital, Out Patient Department.
Received for publication, January 25, 1936

†Personal communication.

strated according to Schilling when 100 differential leucocytes have been enumerated, compared with the Arneith nuclear index after 100 neutrophils have been counted. Schilling's designation of the various cell types is used.

It is essential to bear in mind that the study of a blood smear, by whatever method, is a sampling process, and that the results obtained from the sample will be valuable in direct proportion to the accuracy with which they represent conditions within the patient's peripheral blood circulation. From this point of view, the manipulation of the drop of blood from the moment it is first shed until it is ready for microscopic study is extremely important. The original cell distribution must be altered as little as possible. Furthermore, the cells actually studied, since they constitute but a fraction of those in the entire smear, must be truly representative of the smear as a whole. To insure this, a preliminary series of approximate ratios between cell types from widely separated parts of the smear is necessary, and these approximate ratios will serve as a reliable check against the final detailed results.

ANEMIA LABORATORY

Out Patient Department, Harper Hospital

To: _____ Date: _____

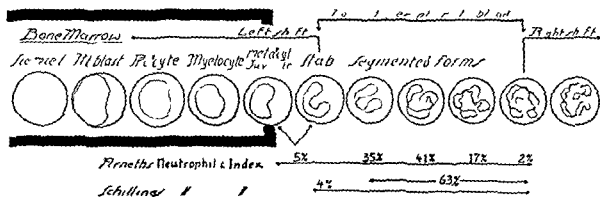


Fig 1—The Arneith and Schilling neutrophilic index in normal peripheral blood (left and right shift)

There are as many ways of making a blood smear and as many methods for the enumeration of the differential elements as there are laboratory textbooks. We use the so called microslide coverslip method for making "margin free" smears as recommended by Schilling.⁴ Only in this type of smear are the erythrocytes well spread and the leucocytes properly distributed. It is essential to keep in mind that the various leucocytes have a different degree of adhesiveness to glass, and it is, therefore, of the utmost importance to have a perfectly clean slide and coverslip. The blood film is made on the convex surface of the microslide.

Making the Blood Film—The blood is taken from the fingertip, which is wiped off sharply with ether, not alcohol, to make it hyperemic. The fingertip is punctured about 5 mm deep with a lancet (7 mm long and 3 mm in width, Eimer & Amend, New York). The first drop oozing from the puncture or upon very slight pressure is discarded (if no blood platelet count is wanted) with the aid of a clean gauze sponge. The second small drop is picked up with the edge of a No 2 coverslip, 18 mm size. The coverslip is put down about one half inch from the end of the microslide at an angle of 45°. After allowing the blood to spread along the coverslip edge by adhesion, the spreader is pushed toward the

other end of the slide with an even, quick, and sure movement, so that the blood follows without any crushing. An average satisfactory preparation is made from a drop that gives a smear extending across one-third to one-half of the slide.

Although the making of a blood smear in this manner is somewhat troublesome, the flexibility of the spreader which fits the slide closely is not only a great asset in obtaining a smear of uniform thickness, length, etc., but also prevents accumulation of myeloid elements at the margin or lymphoid cells in the central part of the film.

After the smear has been properly stained and dried, it is covered with a thin film of immersion oil. The slide is then placed upon the microscope table and with the low power objective, the usable area of the blood film is determined.

Determination of the "Usable Area."—The term "usable area" is defined as that part of the smear which shows all cells evenly distributed. The erythrocytes touch or slightly overlap each other on one extreme vertical margin and on the other they should not be more than one mean cell diameter apart.* Further, the white cell elements should be well stained to make them readily distinguishable under low power. The area about the middle part of the spread should be about 2 cm. long. With the mechanical stage vernier these extreme vertical margins (as defined above) of the area are determined and marked in order that a restudy of the area may be possible at any time.

A survey of the area is first made with the low power objective in order to gain a general idea of the relationship between the myeloid and the lymphoid elements and a knowledge of their distribution throughout the area. Then the relative average ratio is calculated. For this particular procedure the eyepiece is fitted with a device that divides the microscope field into 2, 4, or more sections.

Determination of the "Relative Average Ratio" (R.A.R.).—Within the usable area the leucocytic relative average ratio (i.e., myeloid-lymphoid ratio) is determined. The normal ratio is 2 to 3 neutrophils to 1 lymphocyte. It must be pointed out that the myeloid part of this ratio includes also the elements coming from the reticuloendothelial system. The whole area is carefully and systematically gone over with the low power objective in such a manner that repetition of any section is avoided. About 20 to 25 individual fields which show a leucocytic ratio approximate to that gained from the general survey, are studied and the cells comprising each group (myeloid or lymphoid) are counted and tabulated in separate columns.

For example:

	MYELOID	LYMPHOID
Field 1	6	2
Field 2	12	4
Field 3	15	5

If there is any doubt as to the identity of a cell it should be determined by using the oil immersion objective. No cells which have been mechanically injured may be counted. From the data obtained, the relative average ratio per low power field is determined by adding the columns and dividing by the number of fields studied.

*This ideal "usable area" sometimes cannot be obtained when the erythrocyte concentration is less than 2 ml. per c.mm. and marked aniso-poikilocytosis is present. The vertical margins should then enclose only that area in which the red cells are not more than one and three times of the mean diameter apart.

Enumeration of the percentage relationship of the differential elements (Schilling index) After the relative average ratio has been determined, the actual enumeration of the percentage relationships of the differential elements, or the "internal ratios," begins. Starting at the upper edge of one vertical margin of the usable area, the low power objective is moved an entire vertical length and, keeping the ratio in mind, fields are chosen in which the ratio of myeloid to lymphoid cells approximates the relative average ratio or in other words in which the relationships of cell types approximate the average distribution of cell types in the whole area. The leucocytes in the chosen fields are counted and tabulated according to type in individual columns. The slide is moved several microscopic fields in a horizontal direction and again the objective is moved across the blood smear in the described manner, no field being studied twice.

When 200 leucocytes have been counted the myeloid lymphoid ratio of the cells should be calculated. If care has been exercised, no difficulty will be encountered when reaching the 200 cell limit in having the "external ratio" dovetail with the relative average ratio. Although 200 cells represent the minimum number from which the percentage relationships of the differential elements may be calculated. Shilling and others recommend the counting of 300 to 400 cells. If the internal ratios (differential count) have shifted considerably after the enumeration of the second 100 cells from the number of the first 100 cells an additional 100 or 200 cells are necessary to stabilize the internal ratios. The percentage relationship of all the differential elements may be calculated by simply adding each group separately and dividing by the total number, 200, 300, etc. It does not matter how many fields or how much of the entire area has been covered if at the end of the first 200 cells the internal ratios are quite stable and the "external ratio" dovetails with the relative average ratio found by going over the entire usable area. For example if the relative average ratio was found to be 16 to 1 for the usable area, the external ratio should be the same (see Table I 1 and 2)

TABLE I
R A R 16 to 1

NUMBER OF ENUMERATION	CELLS COUNTED									EXTERNAL	
		M	J	ST	SGM	EOS	BAS	MONO	LYM	RATIO	
1	100	0	6	9	44	2	0	4	35	18 to 1	} 16:1
2	100	0	3	8	40	2	2	5	40	14 to 1	
3	100	0	4	10	40	2	1	5	38	16 to 1	
4	100	0	5	9	44	2	1	2	37	17 to 1	
Total	400	0	18	36	168	8	4	16	150	65 to 4	
	Average	0	4.5	9	42	2	1	4	37.5	16 to 1	

In Table I, the external ratio at the end of the final calculation dovetails at the end of 200 cells but the internal ratio differs from the ratio of the first 100 cells. Since this is an unavoidable variation, 200 cells is the minimum number to be counted before any calculation is made.

The final percentages are reported as whole numbers. If fractions occur in either differential group, these must be absorbed first within the myeloid lymphoid group in which they occur, by adding the fraction to that cell type that

justifies favoritism not only from a technical but also from a clinical point of view. In other words, the cell types favored or disfavored should be judged from experience gained from the study of many ratio groupings, and from the knowledge of the importance of these technical changes when the differential pattern is applied to the clinical findings.

Rule.—The first absorption is made within the myeloid-lymphoid group in which the fractions occur. Remaining fractions which cannot be absorbed within the cell types of the groups are absorbed into that main group that will either balance the external ratio (A.B., Table II) with the original relative average ratio, or will by the gain not unbalance the ratio more than ± 1 per cent.

TABLE II
EXAMPLE OF ABSORPTION OF FRACTIONS
(THE RELATIVE AVERAGE RATIO IS 4 TO 1)

	PERCENTAGES AT COMPLETION OF COUNT		FIRST ABSORPTION		SECOND ABSORPTION
Myelocytes	0	A	0	80.5	0
Juveniles	5.5		6		6
Stabs	15.5		15		15
Segmented	52.5		52		52
Eosinophiles	3.5		4		4
Basophiles	1.5	B	1.5	19.5	1
Monocytes	2		2		2
Lymphocytes	19.5		19.5		20

With the internal and external ratios balanced the enumeration of the differential is completed. On the final report the reticuloendothelial cells (monocytes) are grouped separately, thus:

Myelocytes	0	} 78%
Juveniles	6	
Stabs	15	
Segmented	52	
Eosinophiles	4	
Basophiles	1	
Monocytes	2%	
Lymphocytes	20%	

Reliable information is at hand as to the relative composition of the leucocytic pattern such as the presence of a neutrophilia or neutropenia, including the degree of "shift."

The "ratio of shift" based upon the R.A.R. is a short method for the determination of significant change within a single leucocytic group. In a case in which the percentage of a certain myeloid cell type is desired, the myeloid elements may be enumerated alone, separating the various types in the Arneth or Schilling (see Fig. 1) fashion and supplying a calculated number of lymphoid cells to make up the total of 100 cells. Or, if an enumeration of the lymphoid elements is desired as outlined by Wiseman,³ a calculated number of myeloid elements is supplied to make up the total to 100 cells. Any group can be singled out for study by merely consulting the R.A.R. Thus, if, for example, the R.A.R. is 4 myeloid to 1 lymphoid cell, it is readily calculated that there must be 80 myeloid cells to every 100 leucocytes counted. Then going over the usable

area systematically, fields are chosen in which there is an average distribution of myeloid cell types. The myeloid cells in these fields are enumerated and tabulated according to type until the calculated number of myeloid elements has been reached. In this enumeration, any of the cell types may be still further subdivided and noted in subcolumns. Thus the "stab group" may be divided according to special morphologic characteristics into two subgroups (A) the "band forms," which represent the normal stage in development from the juvenile to the mature segmented form, and (B) the degenerative "stab forms." In the same manner, other types of degenerative or otherwise abnormal neutrophils may be counted separately. Again the lymphocytes are not counted, but the number required according to the ratio is supplied.

In order that the information obtained with the ratio of shift may be reliable, it is well to count two or three times the calculated number to make sure of a "shift" or the number of abnormal cell types. The greater the number of cells counted, the more certain is the stabilization of the ratios.

SUMMARY

The introduction of Arneth's "nuclear index" and Schilling's "hemogram" into clinical hematology revolutionized the method of differentiation and enumeration of the leucocytes in a blood smear.

The successful application of the differential pattern, "biologic reaction to a specific disease," depends chiefly on the method of enumeration of the leucocytes in a blood smear.

It is of the utmost importance that the applied method incorporate three features: it must (1) supply a constant that insures a uniform relative enumeration of the differential elements, (2) permit duplication of the final differential percentages at any time by any one, and therefore prevent marked fluctuation of the myeloid lymphoid ratio that may nullify the first enumeration, and (3) insure the confidence of the clinician in the value of the leucocyte pattern as a diagnostic and prognostic aid. He may then accept instead of reject the data even though the findings deviate greatly from the expected picture.

The method described includes all of the points stressed, with the R A R as the constant. Once the R A R is properly determined, the method makes possible the study of different types or groups of leucocytes without the recounting of all differential cells in a blood smear. With the R A R information at hand, thus changes between the myeloid lymphoid ratio can be obtained in a short space of time.

For future reference the R A R is engraved upon the glass slide or reported on the laboratory sheet. The method allows a technical error of ± 1 per cent of the external ratio in relation to the relative average ratio (R A R).

REFERENCES

1. Arneth, J. Die qualitat
2. Schilling, V. Ueber die
verschiebung im
Ztschr f klin med B 89 1, 1920
3. Schleicher, E. M. The Value of the Schilling Hemogram in Clinical Hematology, Official
Publ. Soc Clin Lab Techn 1 13, 1934
4. Schilling, V. and Gradowohl, R. B. H. The Blood Picture and Its Clinical Significance
St. Louis, 1929, The C. V. Mosby Co.
5. Wiseman, B. K. The Origin of the White Blood Cells, J. A. M. A 103 1524, 1934

Dr. W. Klinkhardt
Beachtung der neutrophilen Kern
aktische Erfolge dieser Methode,

A MOUSE BOX FOR OPERATING ON THE TAIL*

ARNOLD J. GELARIE, M.D., NEW YORK, N. Y.

IN GIVING injections intravenously into the tail of the mouse, or cutting the end of the tail in order to obtain a blood specimen, particularly when this has to be done at frequent intervals, difficulty arises when trained assistance is not at hand.

The box herein described seems to be very suitable and has given great satisfaction for many years in handling thousands of mice without assistance. Fig. 1 is self-explanatory. It consists of a slide box of the usual type, rectangular in shape, with the approximate measurements, 12 cm. in length,

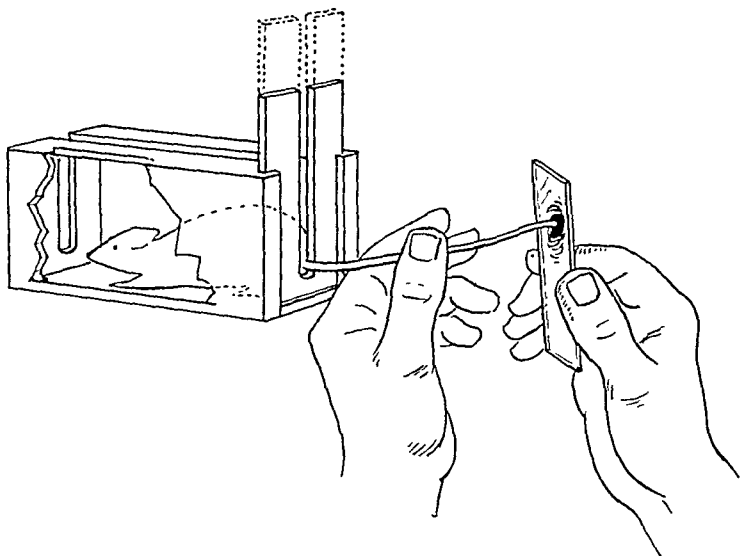


Fig. 1.

6 cm. in width, and 5 cm. in height. One end of the box is fixed, as is the roof; the other end consists of a "door" which is slid into a groove for that purpose. The end, the roof and "door" are slit sufficiently to accommodate the tail, and in such a manner that the slits are central and so continuous with one another. The mouse is allowed to enter the box, or is pulled into it by the tail, which protrudes from the slit in the roof. The sliding "door" is put in place and the tail drawn to one end of the box or the other and held on moderate tension so as to facilitate manipulation and control struggling on part of the animal. In this manner taking of specimens of blood from the tail by snipping off a piece and allowing venous oozing from the tip, or injecting material with a fine needle into the tail veins is greatly facilitated.

54 WEST SEVENTY-FOURTH STREET

*Received for publication, October 19, 1935.

USE OF THE POLAR DIAGRAM IN CHARTING INCIDENCE OF DISEASE*

JOHN W WILLIAMS, CAMBRIDGE, MASS

THE object in using the polar diagram in charting incidence of disease is to afford a means of more readily visualizing monthly and yearly occurrence. That this should be so is logical, since representation of occurrence of disease by a continuous line on a circular chart would throw a picture on the retina which is itself circular. There is not the abrupt discontinuity which is evident in the square and rectangular chart. In learning to tell time, individuals are schooled early in life in grasping the significance of data on the circular diagram.

The diagrams in this paper are almost self explanatory. The total monthly incidence for the states reporting was taken from Supplements 104, 105, 109, and 112 of the U S Public Health Service Reports†. The zones represent the respective incidence for the years studied, the number of cases being plotted on the radii representing the months. The points for the months of each year are connected. The outer curve represents the cumulative cases for the four years. First the number of cases for 1930 were plotted. The resulting curve was then used as the base line and upon it the cases for 1931 were plotted. This in turn was used as the base line for 1932 and so on. By this method termed the "countour method," the lines for the years never cross. This obviates confusion of lines. When data for additional years become available they can be plotted, using the curve of the previous year as the base line. In the diagrams shown, the months have not been corrected for the number of days. With large amounts of data this would be necessary. Since this study is concerned with the trend of the curves and the type figure formed in the several diseases, it was not necessary to correct for population.

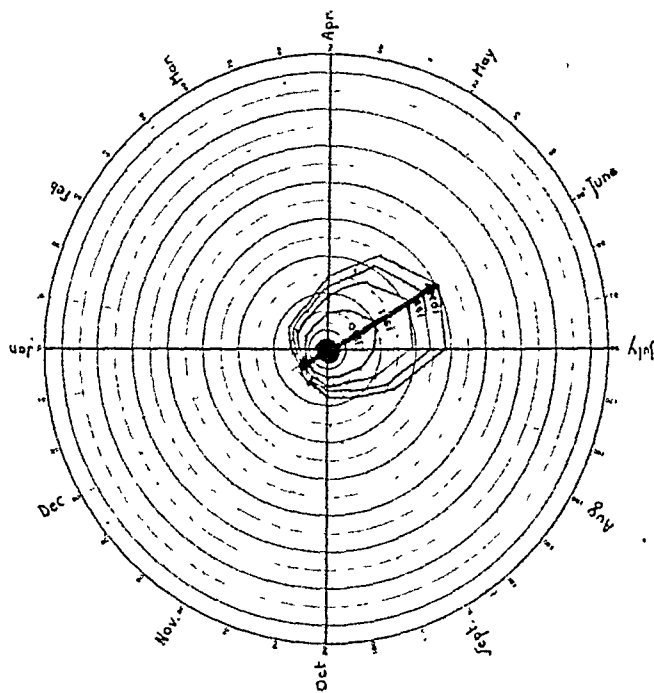
On observing the charts of pellagra, typhoid and paratyphoid and small pox it will be noted that the periods of highest incidence vary. In the diagram of pellagra you will note the hour hand is utilized to denote minimum incidence and the minute hand maximum incidence. In my experience this emphasizes these data to a considerable extent. The curves vary in shape and in the steepness with which incidence rises and falls. Such factors for these and other notifiable diseases will be discussed elsewhere together with variation in incidence over a period of years as illustrated on similar charts. The chart designated pellagra, smallpox and typhoid is an example of plotting yearly

*Contribution No 76 from the Department of Biology and Public Health Massachusetts Institute of Technology

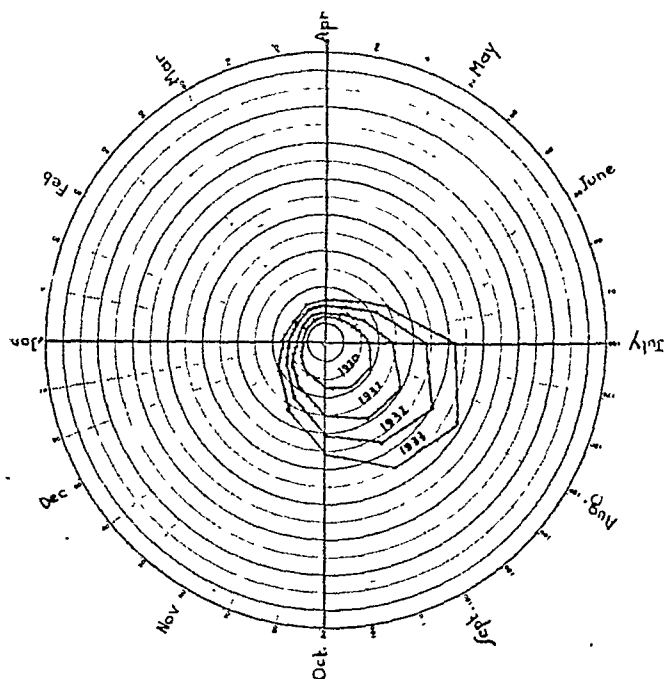
Received for publication November 18 1935

†The incidence is given as typhoid fever for the years 1930 and 1931 and as typhoid fever and paratyphoid fever for 1932 and 1933

PILIAGRA
Cumulative Cases, 1930 to 1933 inclusive
Each Division = 500 cases
Yearly cases represented by zones



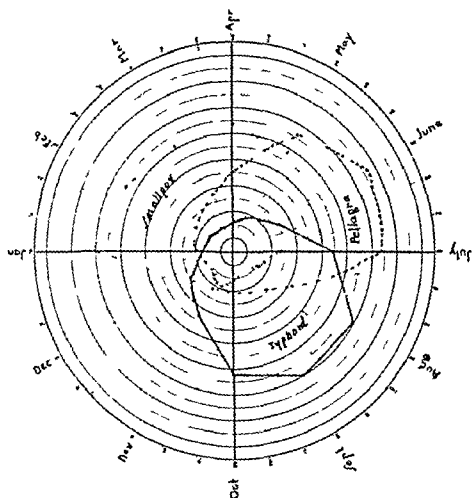
TYPHOID AND PARATYPHOID
Cumulative cases, 1930 to 1933 inclusive
Each division = 500 cases
Yearly cases represented by zones



PELLAGRA, SMALLPOX AND TYPHOID

Year 1931

Each Division - 100 cases

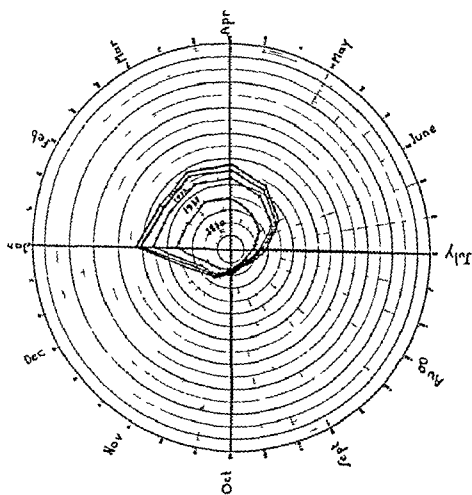


SMALLPOX

Cumulative Cases 1930 to 1933 inclusive

Each division - 500 cases

Yearly cases represented by zones



incidence of three diseases on one chart. It aids in the correlation of their relative frequency and the study of the type of curve of each.

In conclusion, the polar diagram is used in statistical studies, but to my knowledge not in the manner or for the purpose illustrated here. The type of curve and the figure formed and its extent into the quadrants seems for many diseases characteristic. The simplicity of the diagrams is sufficient to illustrate incidence of the disease to the laity. The curves are extremely easy to plot, and additional data can be added for successive years.

REFERENCE

1. Williams, John W.: *Science* 82: 425, 1935.

THE EMPLOYMENT OF OXALATED PLASMA IN THE BROMSULPHALEIN DYE RETENTION TEST*

CARL A. DRAGSTEDT, M.D., PH.D., AND MOORE A. MILLS, M.S., CHICAGO, ILL.

SINCE the development of the bromsulphalein dye retention test as a functional test of the liver by Rosenthal and White,¹ it has gained a rather widespread use because of its relative simplicity when compared to other liver function tests. During the past few years a number of workers have called attention to the fact that so-called reticulo-endothelial blockade may cause marked dye retention. In some studies on reticulo-endothelial blockade, we were desirous of making numerous bromsulphalein tests, and the need arose of making the test more rapid. It occurred to us that, if oxalated plasma could be used, the complete test could be run within fifteen to thirty minutes after obtaining the blood samples, which would greatly facilitate our work. Accordingly, duplicate tests were run on serum in the usual way and on oxalated plasma in a series of experiments to test this possibility. The plasma technic proved so uniformly satisfactory, that inasmuch as we have failed to find any suggestion of its employment in any of the current texts on laboratory and clinical methods available to us, or in the current literature, we felt the present brief report warranted.

The technic we have used is briefly as follows: The dye is injected intravenously in the usual way, 2 or 5 mg. per kilo. At five and thirty minutes samples of blood are withdrawn, using either a clean dry syringe and needle, or a syringe and needle rinsed with isotonic potassium oxalate solution. About 7 or 8 c.c. of blood are collected and introduced into a centrifuge tube containing a drop of saturated potassium oxalate, inverted once, and then centrifuged for fifteen to twenty minutes. The supernatant plasma is removed with a pipette, separated into two tubes suitable for use in the comparator box, one portion being alkalized with sodium hydroxide. The readings are

*From the Department of Physiology and Pharmacology and the Department of Pathology, Northwestern University Medical School.
Received for publication, November 21, 1935.

made in the usual way. In 20 duplicate or comparison readings on serum and plasma in which the percentage dye retentions ranged from 50 (five and thirty minute sample) to 80-60, comparable readings were obtained in every case. As a matter of fact in addition to the considerable saving in time, the following advantages of the plasma technique became apparent, additional blood studies such as red cell counts, volume index, etc. (see Osgood et al.²) can be made on the same sample, and there is usually a larger amount of available plasma after centrifuging a given quantity of blood than there is serum from a similar amount, unless a considerable time is allowed for contraction of the clot.

In addition to the above, we have employed a further modification of the test which has proved serviceable, whenever, for example, shortly after an intravenous injection of India ink for purposes of blockade, or in the accident of some hemolysis, the plasma is not perfectly clear. This consists of precipitating the proteins in the plasma with two volumes of acetone, centrifuging for five minutes and making the dye reading by alkalinizing the clear supernatant fluid. This technique is used in the rose bengal test. Allowance is made for the dilution and the readings made in the usual way. In our hands, the readings after acetone precipitation have tended to be about 5 per cent lower than corresponding readings on the unchanged plasma when the retention is marked. Traces of dye are, however, very readily recognized by this procedure. This technique is not suitable for routine use.

In conclusion, we have found the use of oxalated plasma for the bromsulphalein retention test accurate, simple and rapid, less likely to fail than the serum technique, and advantageous in that other blood studies can be made on the same samples of blood used for the test. In addition, acetone precipitation of the plasma proteins has been found to be efficacious in clearing the plasma of ink and other blocking agents, enabling the test to be run immediately after blocking procedures.

NOTE.—Since submitting this paper for publication, it has come to our attention that Pratt and others³ have used plasma samples for bromsulphalein dye readings. No record of comparison with identical serum samples is given.

REFERENCES

- 1 Rosenthal, S. M., and White, D. C. Clinical Application of the Bromsulphalein Test for Hepatic Function. *J. A. M. A.* 84: 1112, 1925.
- 2 Osgood, F. F., Haskins, H. D., and Trotman, F. E. Uniform System of Hematologic Methods for Use With Oxalated Venous Blood, *J. LAB. & CLIN. MED.* 18: 476, 1931.
- 3 Pratt, T. W., Vinlandingham, H. W., Tilley, L. I., Nelson, T. M., and Johnson, L. O. Studies of the Liver Function of Dogs, *Am. J. Physiol.* 102: 148, 1932.

AN IMPROVED SYSTEM OF FILING SURGICAL REPORTS AND SLIDES*

W. L. ROBINSON, B.A., M.B., TORONTO, CANADA.

BECAUSE of the steady increase in the number of blocks being put through from each surgical specimen, we have been obliged to change our filing system from that of the card with the slide attached to the present one which consists of an envelope containing a slide holder, and copy of the report (Fig. 1).

The envelope is a standard commercial one measuring 4 inches by 6¼ inches, made of glazed laid kraft paper, weighing 62 pounds to the ream, and open at one end. The gummed flap is cut off leaving a plain open end.

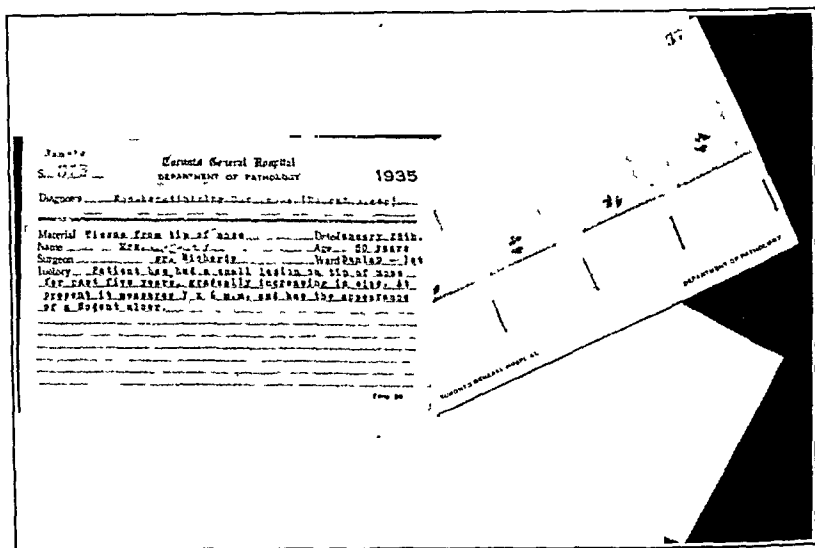


FIG. 1.

The slide holders are made of heavy grayish white sulphite tag, weighing 400 pounds to one thousand sheets of 24 inches by 37 inches. These are cut to 5½ inches by 5⅞ inches. A black line is printed 1½ inches from the bottom to mark the line of fold. At the same time is printed guiding marks for the stapling as well as the hospital name. After folding the card on the black line, five staples are inserted as indicated in Fig. 1, thus making a card 3¾ inches by 5⅞ inches with pockets to hold four slides. This fits quite easily into the 4 inch by 6¼ inch envelope. At the upper left-hand corner of this slide holder, the year is printed with a rubber stamp. On the upper right

*From the Department of Pathology, Toronto General Hospital.
Received for publication, November 13, 1935

hand corner, with an automatic numbering machine, the particular number of the surgical specimen is stamped. The envelope will accommodate three of these slide holders, thus taking care of twelve microscopic sections, besides leaving room for a copy of the report, drawings, and photomicrographs.

The envelope with its contents is now filed in serial order in a steel cabinet of drawers to hold 4 inch by 6 inch cards. They are indexed under the surgeon's name, the patient's name, and also in a disease and region file. For this indexing the Kardex visible records system serves excellently.

THE USE OF LIQUID AIR IN COOLING KNIVES AND OF GELATIN FOR MOUNTING IN FROZEN SECTION TECHNIC*

S CARL WIRCH, M D, ELGIN, ILL.

TWO small changes which appear to be improvements in the usual frozen section technic have been put into operation. The first is to immerse the microtome knife in liquid air in a thermos bottle immediately before using. It takes five minutes to warm up to thawing temperature and has simplified the procedure. Liquid air temperature has no damaging effect on the knife.

The second point is the use of 10 per cent gelatin solution instead of glycerin for mounting. Fewer air bubbles are trapped with this method.

*From the Horse Hospital and Infirmary and the University of Illinois College of Medicine, Department of Physiology.
Received for publication October 26, 1931.

DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

THYROID FUNCTION, Total Blood Fat Determination as an Index of, Chamberlain, C. T., Jacobs, S., and Butler, M. F. *Am. J. M. Sc.* 191: 67, 1936.

The authors do not suggest that determination of the total lipoids should supplant any method used at the present for the study of metabolic disorders; rather it is offered as a valuable addition. They feel that it will afford as much information as will determinations of plasma cholesterol in that it is consistently elevated above the normal 700 mg. per 100 c.c. in hypofunction of thyroid and generally below 400 mg. in hyperthyroidism. In mild cases of hyperfunction it is apt to be between 400 and 500 mg. at which level the diagnostic import is questionable. It is not materially affected by any other endocrine gland disorders, except diabetes, as long as the thyroid is not demonstrably involved.

It is exceptionally of value in those patients for whom the determination of the basal metabolic rate is not feasible.

The depression of the basal metabolic rate following administration of iodine, such as Means has shown to be a routine diagnostic test for hyperthyroidism, is paralleled by the increase of total lipoids. Conversely, its failure in response to iodine is regarded as of significant negative value.

CREATINURIA, of Infancy and Childhood, Marples, E., and Levine, S. Z. *Am. J. Dis. Child.* 51: 30, 1936.

A study of physiologic creatinuria and the influence of extraneous factors on such creatinuria was made on eight normal infants aged from three weeks to seven months. The composite data were collected from a total of one hundred and twenty-nine days. The results may be summarized as follows:

All the infants excreted creatine in the urine, the creatine coefficients (mg. of nitrogen per kilogram of body weight in twenty-four hours) varying from 0.3 to 5.14. The average value for infants over one month of age was 3.89. The coefficients ranged from 3.74 to 5.78, with an average of 4.93. The total creatine coefficients (creatinine plus creatine) ranged from 4.82 to 10.5 with an average of 8.86 for the group of infants over one month of age. With few exceptions the total creatine coefficients fell within the range of coefficients for pre-formed creatinine established for adults.

Age appeared to have no influence on the excretion of creatinine within the limited age range studied here (three weeks to seven months). Premature infants excreted practically no creatine, and this was also true for some very young full-term infants.

Creatinuria of infants could be diminished by feeding diets low in protein but of adequate caloric value. It could be increased by feeding diets high in protein. The excretion of creatinine was not materially affected by these changes in diet.

Infants have a low tolerance for ingested creatine. From 55 to 65 per cent of the amount ingested was excreted in the urine as creatine in the first twenty-four hours and 63 to 82 per cent in forty-eight hours.

The ingestion of amino-acetic acid increased the spontaneous creatinuria of infants but had no effect on the excretion of creatinine. Only a small fraction of the ingested amino-acetic acid was represented in the extra creatine excreted, and there was no specific quantitative relationship between the amount of amino-acetic acid ingested and the extra creatine excreted.

The effect of withdrawal of available amino-acetic acid from the body by diverting it to the formation of hippuric acid for detoxification of benzoic acid was studied. The ingestion of sodium benzoate causes an initial increase in creatinuria, due perhaps to the increased protein metabolism caused by the benzoate, followed by a decrease to below the foreperiod level.

CEREBROSPINAL FLUID, Comparative Dextrose Content of Lumbar and Cisternal, Levinson, A., and Cohn, D J. Am J Dis Child 51 17, 1936

A study has been made of the comparative dextrose content of the lumbar and the cisternal cerebrospinal fluid obtained from nonmeningitic and meningitic patients and from normal dogs.

In nonmeningitic patients the dextrose content of the cisternal fluid was found to be either equal to that of the lumbar fluid or slightly higher. The dextrose content of the lumbar fluid of those patients in whom no pathologic change in the nervous system was found averaged 57 mg per hundred cubic centimeters, and that of the cisternal fluid 60 mg. The results on normal dogs were similar to those on human beings.

In patients with tuberculous meningitis and in untreated patients with meningococcal meningitis, the dextrose content of the lumbar fluid was always reduced. The dextrose content of the cisternal fluid, while usually lower than normal, was not as low as that of the lumbar fluid. In some instances the dextrose content of the cisternal fluid was within the normal range, even though dextrose was absent or very little was present in the lumbar fluid. In patients with meningococcal meningitis after serum treatment the values for dextrose in the cisternal and lumbar fluid were much closer to the normal than the values found for untreated patients, and the differences between the levels in the two fluids were smaller.

In the majority of a limited series of fluids from patients with nonmeningococcal purulent meningitis, particularly of the pneumococcal and streptococcal types, there was a very low amount of dextrose or none in the cisternal as well as in the lumbar fluid.

Determinations of the dextrose content of the lumbar fluid are more reliable for the diagnosis of meningitis than determinations made on the cisternal fluid because the dextrose content of the cisternal fluid is seldom reduced as greatly as that of the lumbar fluid.

TUBERCLE BACILLUS. A Glycerol Free Medium for, Henley, R R. Am J Tuberc 32 724, 1936

During the course of studies of the biology of the tubercle bacillus in these laboratories, a medium has been devised which contains neither amino acids nor glycerol, but which nevertheless affords an abundant growth of the human type of the organism. Since a medium of this type may be of value to those engaged in the study of the metabolic processes of the tubercle bacillus the following notes are presented:

Ammonium malate	12.53 gm
Dipotassium phosphate	1.80 gm
Sodium citrate	0.75 gm
Magnesium sulphate	1.50 gm
Ferric citrate, scales USP VIII	0.30 gm
Dextrose, of the grade known as Cerclose	80.00 gm
Water distilled, to make	1 000.00 cc

PREPARATION OF MEDIUM

The ammonium malate is prepared by dissolving 10 gm of maleic acid (grade known as "Food Pure") in about 500 cc of distilled water. Ammonium hydroxide, CP is added to the solution until this is neutral to litmus. The other ingredients of the medium are then added in the amounts and order given in the formula. Each compound is dissolved completely before the next is added. The required amount of ferric citrate is dissolved in hot water before it is added to the solution.

The complete medium is placed in 300 cc Durham flask, 100 cc per flask, and is sterilized by heating under 10 to 12 lb under steam pressure for twenty minutes or alternately

tively, by heating in flowing steam for thirty, twenty, and fifteen minutes, respectively, on each of three successive days. Heating causes a lowering of the pH value; hence, excessive heating must be avoided. The pH value of the sterilized medium should not be lower than 6.0. It is possible and desirable so to prepare and sterilize the medium that the pH will be not lower than 6.4 nor higher than 7.

The sterilized medium is inoculated with seed from vigorously growing cultures not more than fourteen days old, and the inoculated medium is incubated at 37.5° C.

Growth starts slowly, requiring four weeks or longer to reach 0.50 gm. dry weight of bacilli per 100 c.c. of culture medium, but, once started, it continues steadily and reaches a maximum about the eighth week. The greatest weight thus far obtained is 1.82 gm. per 100 c.c. culture fluid, and the average weight of bacteria in mature cultures is approximately 1.60 gm. per 100 c.c. As with other media, the weight of bacteria reaches a maximum and then tends to decrease. Growth is not always uniform, since differences in weight are frequently found between two or more cultures of the same age and batch.

BLOOD, Puncture of Spleen in Diseases of, Perles, S. Paris méd. 25: 217, 1935.

The following is the normal "splenogram":

Neutrophilic polynuclears	20 to 30%
Eosinophilic polynuclears	1%
Basophilic polynuclears	rare
Typical lymphocytes	50 to 60%
Medium mononuclears	10 to 20%
Monocytes	5 to 10%
Plasmocytes	1 to 2%
Normoblasts	0.20%
Naked nuclei	numerous
Background, thick, sometimes granular	
Erythrocytes, normal	
Platelets, very numerous	

ANEMIA, Absorption, Storage, and Elimination in Experimental, Hahn, P. F., and Whipple, G. H. Am. J. M. Sc. 191: 24, 1936.

Reserve iron storage in the dog can be exhausted during two to three months by a continuous anemia with hemoglobin level maintained at one-half to one-third of normal. Given this elimination of reserve iron we may attempt to trace iron given by mouth or vein.

A rapid turnover of iron is the conspicuous feature of the experiments dealing with iron given by mouth and short feeding experiments (one to two days) give no evidence of any iron store in the liver. As the iron feeding experiments are lengthened we see a variable accumulation of iron in the liver, but the amounts are small and a very rapid appearance of the iron in matured hemoglobin is the conspicuous feature.

The importance of blood-free organs is stressed, and other method difficulties are discussed.

Parenchyma iron of various blood-free organs is relatively a constant in these dogs. Liver, kidney and pancreas average 1 to 2 mg. Fe per cent. The lung is a little higher, or 3 mg. Fe per cent. The spleen is still higher, or 5 to 6 mg. Fe per cent. The red marrow apparently has the highest level of parenchyma iron, an uncertain figure but probably in excess of 10 mg. Fe per cent. The striated muscles actually rate with the liver and kidney, although the average for total contained iron is 3.1 mg. Fe per cent. About 1.6 mg. Fe is muscle hemoglobin iron, leaving the parenchyma iron as 1.5 mg. Fe per cent.

Muscle hemoglobin iron and muscle parenchyma iron are inviolate stores of iron which are not drawn upon no matter how great is the emergency due to anemia. Conversely, no surplus iron can be demonstrated in this tissue where iron is given intravenously.

Iron depletion can be carried to a point where there is almost a complete cessation of hemoglobin production in a standard dog on a diet very poor in iron.

Intravenous iron in the dogs given will result in large storage in the liver and spleen, 55 to 70 per cent of the total Fe given. The authors are not prepared to say where the remaining iron is to be located in the body tissues or fluids but it certainly is not eliminated.

ANEMIA Iron and Its Utilization in Experimental, Whipple, G. H., and Robschert-Robbins, F. S. *Am J M Sc* 191 11, 1336

Iron given intravenously to these standard anemic dogs, in large or small doses, will be returned quantitatively as newformed hemoglobin on the basis of 10 mg Fe = 3 gm hemoglobin.

When iron is given by mouth to these standard anemic dogs the influence on hemoglobin production is not proportional to the amount of iron administered. An optimum dose of iron (40 mg Fe per day for two weeks, or 360 mg) will give a net output of about 55 gm hemoglobin, and this means about a 35 per cent utilization of the iron given. With larger doses the hemoglobin output increases a little, and with 400 mg Fe daily the net output would average about 95 gm hemoglobin, or about a 5 per cent utilization of the iron given by mouth.

Iron contained in salmon bread under certain conditions may be utilized by these anemic dogs up to about 40 per cent to produce hemoglobin. Iron in the normal liver when fed may show a 45 per cent utilization of this tissue iron.

When liver feeding is combined with iron feeding, there is a definite summation effect.

When intravenous iron in large doses is combined with liver feeding or iron by mouth, there is no evidence of summation.

This probably means that the top functional capacity of the body to produce hemoglobin has been reached, about 10 gm hemoglobin output per day.

Iron salts are utilized with equal facility by the dog to produce hemoglobin when given by mouth in the ferrous, ferric or reduced state. The determining factor is the amount of the iron metal.

The Eck fistula and splenectomy lessen the capacity of the anemic dog to conserve and utilize iron given by vein to produce new hemoglobin.

SILICOSIS Clinical Observations on the Pulmonary Blood Flow in, and Other Fibrotic Conditions of the Lungs, Miller, H. R. *Am J M S* 191 334, 1331

The fibrosis in pulmonary disease assumes two main forms: (a) A diffuse type involving the vast capillary alveolar bed associated as a rule with pulmonary hypertension and observed in emphysema or in obstruction to the lesser circulation (mitral stenosis, kyphoscoliosis, etc.), and (b) a circumscribed or 'patchy' fibrosis involving the parenchyma and encountered in tuberculosis, carcinoma, etc. In silicosis the diffuse type is present early and the second or patchy form appears much later.

From a vascular point of view the heart and lungs are one organ, the lungs represent an enormous vascular sponge of unified vessels stemming from the heart and surrounded by air sacs through which gases are exchanged between the circulation and the outside atmosphere. With this concept in mind it is not surprising to find that pulmonary disease and conditions associated with marked fibrosis remain extravascular as a rule and that when the peripheral vascular lung bed becomes involved a concomitant state of pulmonary hypertension is the rule. The heart if at all affected is drawn into the picture secondarily either (a) by developing hypertrophy of the right ventricle as a response to this hypertension in some cases accompanied by the terminal picture of so-called right heart failure, or else (b) the heart responds by altering its volume output as a compensatory reaction to an increase in the A-V difference; this difference following as a result of an interference in gas exchange through a pathologically altered alveolar wall.

Unless cardiac failure (congestive failure) intervenes, the rate of pulmonary blood flow remains normal in both arterial and venous circuits in all pulmonary fibrotic conditions. To

this there are few exceptions. As in other fibrotic conditions, the rate of pulmonary blood flow in silicosis is not deranged unless heart failure sets in.

The rate of pulmonary blood flow is retarded in the venous channels in the presence of cardiac congestive failure.

In a few instances of pulmonary fibrotic states the velocity of blood flow was retarded in the pulmonary arterial channels. Here the fault would appear to be in the alveolar membrane.

CANCER, The Reaction of Spontaneous Mouse Tumors to Cystine Disulphoxide, Staff of Lankenau Hospital Research Institute. Am. J. Cancer 26: 554, 1936.

The daily subcutaneous administration of 0.0085 gm. cystine disulphoxide to mice with spontaneous tumors was accompanied by tumors of lesser maximum size than those in untreated controls, by a lesser maximum percentage increase in volume, by a slower attainment to a given volume, and by a lesser percentage of tumors attaining a given volume. The tumors of the disulphoxide injected mice were watery and diffuse; they had fewer cells per unit measure and their nuclei tended to be larger than those of controls. The anemia of malignancy tended to be enhanced. Body weights were essentially unaffected. A tendency for the injected mice to live longer than the controls was exhibited. There was no overt tissue pathology.

The consistency in and between these data leads to the conclusion that proliferation growth of malignant spontaneous tumors of the mouse is retarded, as is that of all other expressions so far encountered, by partially oxidized derivatives of the sulphhydryl group as here represented by cystine disulphoxide.

Correspondence

Two twelve-month Residenceships in Allergy are available with Doctors Vaughan and Graham in Richmond, Virginia, for properly qualified young physicians. Appointments are made semiannually. Completion of an internship, either rotating or in Internal Medicine in a nationally recognized teaching hospital, is a prerequisite.

The course of training includes practical experience in the study and treatment of the allergic diseases as well as general training in the practice of internal medicine. Rooms and offices are provided but not board or laundry. In lieu of the latter, there will be a salary of seventy-five dollars per month.

Residents will be required to devote a portion of their time to investigative work (clinical and laboratory) in the field of allergy.

Inquiries or applications should be made to Dr. Warren T. Vaughan, 201 West Franklin Street, Richmond, Va.

The Journal of Laboratory and Clinical Medicine

WARREN T. VAUGHAN, M.D., Editor
808 Professional Building
Richmond, Va.

ASSOCIATE EDITORS

Pharmacology

DENNIS E. JACKSON, M.D.
University of Cincinnati, Cincinnati

Tuberculosis

GERALD B. WEBB, M.D.
Cragmor Sanatorium, Colorado Springs

Clinical Pathology

W. C. MACCARTY, M.D.
Mayo Clinic, Rochester, Minn.

T. B. MAGATH, M.D.
Mayo Clinic, Rochester, Minn.

Biochemistry

VICTOR C. MYERS, Ph.D.
Western Reserve University, Cleveland

Experimental Medicine

RUSSELL L. HADEN, M.D.
Cleveland Clinic, Cleveland

Immunology

JOHN A. KOLMER, M.D.
Temple University, Philadelphia

ROBERT A. KILDUFFE, M.D.
Atlantic City, N. J.

Internal Medicine

GEORGE HERRMANN, M.D.
University of Texas, Galveston

Bacteriology

M. H. SOULE, Sc.D.
University of Michigan, Ann Arbor

Metabolism

CLIFFORD J. BARBORKA, M.D.
Northwestern University, Chicago

Surgery

DEAN LEWIS, M.D.
Johns Hopkins University, Baltimore

VOLUME 21
OCTOBER, 1935—SEPTEMBER, 1936

ST. LOUIS
THE C. V. MOSBY COMPANY
1936

Copyright, 1936, By the C. V. Mosby Company
(All rights reserved)

Printed in U. S. A.

Press of
The C. V. Mosby Company
St. Louis, Mo.

INDEX TO VOLUME 21

AUTHORS INDEX

In this index following the author's name the title of the subject is given as it appeared in the Journal. Editorials are also included in the list and are indicated by (L)

A

- ADAMS C C (See ANDER KAMMEIER AND ADAMS) 340
ALEXANDER R C (See McEWEN ALEX ANDER AND BUNIM) 453
— (See McEWEN BUNIM AND ALEXANDER) 465
ALLEN ALEXANDER J (See SANIGAR AND ALLEN) 969
ANDES JEROME E KAMMEIER R H AND ADAMS C C Studies of plasma proteins and cholesterol 340
ARMISTEAD D B (See KORBES NEALE HITE ARMISTEAD AND RUCKER) 1036
ARMSTRONG E L AND KUDER M L Limitations of colorimetric analysis by present methods 181
ASHBY WINIFRED Prolonged preservation of antigenic specificity of sheep corpuscles associated with production of a marked increase in resistance to hypotonic solutions 943
AUERBACH OSCAR (See FRIEDMAN AND AUERBACH) 93

B

- BALYEAT RAY M SEYLER L EVERETT AND OUTHIER VIRGIL Iodized oil 187
BARDWELL KATHLEEN (See BAUMBERGER AND BARDWELL) 179
BAUMBERGER J PERCY AND BARDWELL KATHLEEN New method for determination of cutaneous capillary blood pressure 179
BEEBE RICHARD T AND HANLEY EDWARD P A method for measuring fragility of erythrocytes in salt solution 833
BELK WILLIAM P Autohemagglutinin 697
BENNETT A L A synchronous motor clock time clock 757
BENSLY F H The interference of nitrites in detection and estimation of urobilinogen in urine 119
BISGARD J DEWEY (See SHARPE AND BISGARD) 347
BLACK L A (See FABER AND BLACK) 1069
BLALOCK ALFRED AND BURWELL C SIDNEY Thoracic duct lymph pressure in congenital cordis 296
BOERNER FRED LUKENS MARGUERITE AND GILMAN R L A study of relative sensitivity and specificity of Kolmer Wassermann Kahn and Lagle precipitation tests 952
BOWEN BYRON D AND LOCKIE L MAXWELL Chronic atrophic arthritis the effect of a high carbohydrate diet and insulin on the symptoms and respiratory metabolism 505
BOWMAN RUSSEL O A rapid method for routine serum protein determination 1092
BOYD ILDON M The estimation of phospholipid content of white blood cells 97
BRAKFIELD J L (See CRANE AND BRAKFIELD) 1203
BRAM ISRAEL Quinine test for hyperthyroidism 123
BRODY HENRY SMITH LAWRENCE W AND WOLFF WILLIAM I Blood grouping in infectious diseases 705
BROOKS CLYDE A new method and a new pipette for blood sedimentation 971

- BLAIM JOSEPH J (See McEWEN ALEX ANDER AND BUNIM) 453
— (See McEWEN BUNIM AND ALEXANDER) 465
PURGL W E ORTH O S NEILD H W KROUSE R AND WICKWIRE G C The cause and significance of electro negativity of active living tissue 1162
BURNS J H (See SCHMIDT EASTLAND AND BURNS) 13
LURWELL C SIDNEY (See BLALOCK AND LURWELL) 296
BUTTS C EARL (See HARNF AND BUTTS) 869
BUNBAUM EDWARD AND GREENWALD CHARLES K chemical study of alum diphtheria toxin precipitate 157

C

- CARTER J BAILEY AND TRAUT EUGENE F Auricular flutter with 1:1 response 670
CASILLI ARTURO R A new microreaction for serodiagnosis of syphilis 1201
CHAPMAN GEORGE H (See RAWLS AND CHAPMAN) 49
CHIFF GRAHAM Microdetermination of sodium by urinary zinc acetate methiod and titration of uranium with cadmium as reductant 1195
CLARKE CARL DAVE Technique of molding and casting for medical sciences 68
CLIVENKO DAVID ROBERT Modification of hematopoietic function in rabbit by certain cyclic compounds 913
COCA ARTHUR F (See TOWNSEND AND COCA) 729
COHN MAURICE L (See CORFFER AND COHN) 423
CORFFER H J AND COHN MAURICE J Mechanical device for preparing fine suspensions of tubercle bacilli and other microorganisms 158
COULTER JOHN S Home treatment of chronic arthritis by physical therapy 497
COUSINS RICHARD F (See MOLITCH AND COUSINS) 43
COX W V HAWKINS J W AND ROBERTSON H F Method of estimating put on dogs 192
CRANE W H AND BRAKFIELD J L A modification of Wright's technique for determination of opsonic index 1203
CROCKER WALTER J AND VALENTINE FLORA Hemography in diagnosis of appendicitis based on 500 cases 883

D

- DALFY FRANCIS H (See HEALY DALFY AND SWIFT) 938
DAVID NORMAN A AND LINCOLN RALPH A An inexpensive laboratory timing device 860
DAVIS JOHN STACE JR Protein studies in atrophic (rheumatoid) and hyperatrophic arthritis 478
DAWSON M H (See TAYLOR FERGUSON KARABACH AND DAWSON) 491
— AND TYSON T LLOYD Relationship between rheumatic fever and rheumatoid arthritis 775

- DE'ANDORI, JAMIS H. Effect of hydrogen ion concentration upon the determination of calcium in blood serum phosphomolybdic acid centrifugates, 65
- DEYOUNG, WILLARD. Sources of error in laboratory diagnosis of amebiasis, 1119
- DILL, L. V. The effect of obstructive jaundice on blood platelets of rabbit, 899
- DINGLE, JOHN H. (See NORTON, DINGLE, AND SHERSTONE), 1083
- DODGE, KATHARINE G. (See SUTTON AND DODGE), 619
- DRASTLDT, CARL A., AND MILLS, MOORE A. The employment of oxalated plasma in bromsulphalein dye retention test, 1306
- DUBOIS, DELAFIELD. (See FERGUSON AND DUBOIS), 663

E

- EASTLAND, J. S. (See SCHMIDT AND EASTLAND), 1, 233
- (See SCHMIDT, EASTLAND, AND BURNS), 13
- ELHARDT, W. P. Pain and weather, 1247
- ETTELSON, L. N. (See WOHL AND ETTTELSON), 390

F

- FABER, J. E. JR., AND BLACK, L. A. The influence of physiologic salines in complement fixation reactions, 1069
- FALK, K. GEORGE. (See VALENTINE AND FALK), 257
- FALKER, I. DAVID. (See KOOPMAN AND FALKER), 308, 312, 808
- FELSEN, JOSEPH. Intestinal illuminator, 923
- FERGUSON, A. B. (See TAYLOR, FERGUSON, KASABACH, AND DAWSON), 491
- FERGUSON, JOHN A. (See WELCH, MURDOCK, AND FERGUSON), 1264
- , AND DUBOIS, DELAFIELD. Observations on pH of clotting and citrated blood, 663
- FETTERMAN, GEORGE H., AND LERNER, HARRY. A fatal case of tularemia pneumonia with associated ileitis, 1157
- FINE, JOSEPH. Determination of fibrin by biuret method, 1081
- FORBES, J. C., NEALE, R. C., HITE, O. L., ARMISTEAD, D. B., AND RUCKER, S. L. Studies on effect of a high-sulphur low-carbohydrate diet in chronic arthritis, 1036
- FOY, HENRY. A simple, efficient, and inexpensive device for drying pipettes and other laboratory glassware, 435
- FRAENKEL, E. M., AND PULVERTART, R. J. V. Prevention of anaphylactic shock due to horse serum by injection of B.C.G., 359
- FRIEDMAN, GEORGE, AND HOLTZ, EDWARD. Behavior of eosinophiles in rheumatic fever, 225
- FRIEDMAN, MAX M., AND AUERBACH, OSCAR. An improved Congo red test for amyloidosis, 93

G

- GALE, C. K., AND MILLER, DAVID. Bactericidal action of short and ultrashort waves, 31
- GALLUP, WILLIS D. A convenient food cup for rats, 1194
- GARDNER, RAYMOND E. (See MORRISON, GARDNER, AND REEVES), 822
- GARRETT, ORVILLE W. (See PRICKETT AND GARRETT), 942
- GELARIE, ARNOLD J. A mouse box for operating on the tail, 1302
- , A new, one-minute method for staining of spirochetes, spirilla, spermatozoa, and related organisms, 1065
- GILMAN, R. L. (See BOERNER, LUKENS, AND GILMAN), 952
- GLASSBERG, B. Y. Incidence of nondiabetic glycosuria, 152
- , Safely induced fever therapy in a diabetic individual, 820

- GOLD, HERMAN. Studies on anthrax, 134
- GOOSMANN, CHARLES. Dark-field illumination in diagnosis of tuberculosis and malaria, 121
- GOWEN, G. HOWARD. Fluctuations in basophilic aggregation counts with meteorologic alterations, 677
- GRAESSER, JAMES B., AND ROWF, ALBERT H. Administration of epinephrine by inhalation, 1134
- GREEN, LEO. (See WILLIAMS AND GREEN), 785
- GREENE, JAMES A., AND WARD, MURIEL. Frequency and significance of changes of expiratory chest volume during routine measurement of basal oxygen consumption, 1231
- GREENBLAC, DAVID M., AND MIROLUBOVA, TATIANA N. Modification in colorimetric determination of plasma proteins by Folin phenol reagent, 431
- GREENSPAN, A. (See HAZEN AND GREENSPAN), 1185
- GREENWALD, CHARLES K. (See BUNBAUM AND GREENWALD), 157
- GRIFFITH, J. Q., JR., ROBERTS, ELLA, AND JEFFERS, W. A. A staining technic for blood in spinal fluid, 1208
- GROAT, WILLIAM A. A general purpose polychrome blood stain, 978

H

- HADEN, RUSSELL L. Organization in study and control of rheumatic diseases, 548 (E)
- , AND WARREN, WIRT A. Accelerating factors in chronic hypertrophic arthritis (osteoarthritis), 448
- HANLEY, EDWARD P. (See BLEEBE AND HANLEY), 833
- HANSCOM, VIRGINIA. (See KOVACS, HARTUNG, AND HANSCOM), 1022
- HANZLIK, P. J. (See LEHMAN, RICHARDSON, AND HANZLIK), 95
- HARNE, O. G., AND BUTTS, C. EARL. Muscle nerve stimulating accessory for Harvard kymographs, 869
- HARTUNG, EDWARD F. (See KOVACS, HARTUNG, AND HANSCOM), 1022
- , The treatment of atrophic (rheumatoid) arthritis with leucocyte concentrate, 536
- HAWKINS, J. W. (See COX, HAWKINS, AND ROBERTSON), 192
- HAZEN, E. L., AND GREENSPAN, A. A study of nonspecific reactions of cerebrospinal fluids with Bordet and Ruelens antigen in complement fixation test for Syphilis, 1185
- , AND MORTILLARO, MARY. A hitherto undescribed microorganism of alkaligenes group, 710
- HEALY, JAMES C., DALEY, FRANCIS H., AND SWEET, MARIAN H. Medical aspects of periodontoclasia and gingivitis, 698
- HELLWIG, C. ALEXANDER. Does calcium neutralize thyroxine? 1131
- HELWIG, FERDINAND C., AND SCHUTZ, CARL BRYANT. Further contribution to liver-kidney syndrome, 264
- HENCH, PHILIP S. The present status of fever therapy in treatment of gonorrheal arthritis, chronic infectious (atrophic) arthritis, and other forms of "rheumatism," 524
- HERRMANN, GEORGE. Renal function tests, 989 (E)
- HESELITINE, H. CLOSE. (See HOPKINS AND HESELITINE), 1105, 1113
- , AND HOPKINS, E. W. Fungicides, 288
- , AND NOONAN, W. J. Fungicides, 281
- HITCHENS, ARTHUR PARKER. Hematologic nomenclature, 173
- HITE, O. L. (See FORBES, NEALE, HITE, ARMISTEAD, AND RUCKER), 1036
- HOFFMAN, WILLIAM S., AND MANSTEAD, JOSEF V. Significance of serum in organic sulphate concentrations in Bright's disease, 380
- HOLTZ, EDWARD. (See FRIEDMAN AND HOLTZ), 225
- HOPKINS, E. W. (See HESELITINE AND HOPKINS), 288

- , AND HILSLETTINI, H. Cross Cultural and morphologic studies of cryptococci and moniliae isolated from vulvovaginitis and oral thrush, 1113
- , AND —. Reliability of filamentation tests in identification of moniliae, 1107
- HUBBETZ, M. CAROLINE. Blood sugar level after prolonged carbohydrate feeding, 1113
- HUNTER, CHARLES A. A rapid method for preparing antigens for Wassermann reaction, 417
- HUTTON, JAMES H. Recent advances in endocrine diagnosis and treatment, 736
- HANDMAN, OLAN R., AND PUTNAM, TRACY. A method of three color photography, 850

I

- IDE, SOREL, AND IDE, TAMAO. Ide test—the new color test for syphilis, 1190
- IDE, TAMAO. (See IDE, SOREL, AND IDE, TAMAO), 1190
- INGERSOLL, WINIFRED. Hemoglobin values in normal adults over a period of time 787
- IPONS, ERNEST D. Introductory remarks, 445
- ISQUIERO, J. J. On chronic hypertension of nervous origin, 235

J

- JACKSON, D. E., AND JACKSON, HELEN L. Experimental and clinical observations regarding angina pectoris and some related symptoms, 993
- JACKSON, HELEN L. (See JACKSON, D. E. AND JACKSON, HELEN L.), 993
- JAGLENSKI, T. (See MCCULLAGH, JAGLENSKI AND KLOBUCAR), 732
- JAMIESON, W. A. (See POWELL AND JAMIESON), 301
- JEFFERS, W. A. (See GRITTHILL, ROBERTS AND JEFFERS), 1208
- JERKE, LOUIS G. Diagnosis of carcinoma of stomach from a fragment of tumor obtained during routine gastric analysis, 836
- JONES, OLIVER P. Reaction of normoblastic bone marrow to liver extract 335
- JOSLPHSON, BERTIL. Influence of composition of antigen-extracts for some serologic tests on syphilis (M.B.R. II and M.K.R. II) 751
- JUNG, RUTH WESTLUND. The importance of leucocyte counts in phagocytic tests 760
- JUSTER, IRVING R. Normal range of leucocyte count determined weekly over an extended period 376

K

- KAISER, ALBERT D. Influence of tonsils on rheumatic infection in children 609
- KAMPMEIER, R. H. (See ANDES KAMPMEIER AND ADAMS), 340
- KASABACH, HAIG. (See TAYLOR, FERGUSON KASABACH AND DAWSON), 491
- KATZ, H. L. AND NICE, L. B. The relation of nonfilament and filament counts during excitement 1145
- KEIL, H. L. AND NELSON, VICTOR E. Further studies on copper and iron in metabolism 1119
- KELLER, MARGARET. (See MINOT AND KELLER), 743
- KELLEY, MARGARET F. AND SHORT, JAMES J. The alleged variabilities of Kahn and Wassermann reactions from meteorologic changes 910
- KELLY, LEMOYNE C. (See SNYDER TRAEGER, ZOLL KELLY, AND LUST), 541
- KILDUFFE, ROBERT A. Thoughts on the education of the public 330 (B)
- KINARD, F. W. AND VAN DE ERVE, J. Control of mange in laboratory dogs 1203
- KING, JOSEPH T. A ring type sterilizer for use with tissue culture flasks 939
- , A technique for obtaining human blood for tissue culture experiments 940

- KLECKNER, A. L. Bacteriologic studies on fecal streptococci and lactic acid streptococci, 111
- KLEIN, JACOB H. Comparative studies in chlamydias, 1017
- KOOPMAN, JOHN, AND FAIRER, I. DAVID. A more sensitive complement fixation test for gonorrhea, 308
- , AND —. Inadequacy of present complement titrations, 311
- , AND —. Methods for determining erythrocyte permeability, 808
- KOSTER, HARRY, SHAPIRO, ARTHUR, AND POSEN, EDNA. A method for microdetermination of procaine in cerebrospinal fluid, 1096
- KOVACS, JOSEPH, HARTUNG, EDWARD F., AND HANSCOM, VIRGINIA. Constitution and arthritis, 1022
- KRAKFI, JOSEPH, JR. A simple treatment for psoriasis, 1147
- KRAMER, DAVID W. Blood sedimentation rate in diabetes mellitus, 37
- KROUSE, R. (See BURGE, ORTH, NEILD, KROUSE AND WICKWIRE), 1162
- KRUEGER, FREDERICK J., AND MEYER, OVID O. Lymphogranulomatosis (Hodgkin's disease), 682
- KUDER, M. L. (See ARMSTRONG AND KUDER), 181
- KUGELMASS, I. NEWTON, AND SAMUEL, EDNA LOUISE. Animal growth and space restriction, 655

L

- LAKE, MICHAEL. (See TORREY AND LAKE), 1170
- LANDY, MAURICE. (See SPOHR AND LANDY), 650
- LARSEN, NILS P. Pathologic library of gross specimens, 916
- LARSON, HARDY W. Comparison of xylose tolerance with blood urea in nephritic rats, 1010
- LEADINGHAM, ROY S. Sodium citrate—a spirocheticide, 922
- LEDERER, MAX. (See RADWIN AND LEDERER), 1017
- LEHMAN, A. J., RICHARDSON, A. P., AND HANZLIK, P. J. Improved procedures for estimating bismuth in body fluids and tissues, 95
- LERNER, HARRY. (See FEITZMAN AND LERNER), 1157
- LESLIE, ELEANOR I., AND SANFORD HEYWORTH, N. A method of quantitative and qualitative estimation of platelets in their own plasma, 1078
- LINCOLN, RALPH A. (See DAVID AND LINCOLN), 860
- LINTZ, ROBERT M. Red blood cell sedimentation rate in chronic sinusitis, chronic tonsillitis, and dental periapical infections, 1259
- LOCKIE, L. MAXWELL. (See BOWEN AND LOCKIE), 505
- LUETH, HAROLD C. AND SUTTON, DON C. Lung abscess, 1056
- LUKE'S, MARGUERITE. (See BOERNER, LUKENS AND GILMAN), 302
- LUST, F. J. (See SNYDER TRAEGER, ZOLL KELLY, AND LUST), 541

M

- MAJOR, RALPH H. Intranasal application of insulin 278
- MAITBY, ALICE B., AND WILLIAMS, JOHN D. Attempts to apply acetylene method of determining cardiac output to dog 354
- MANSFIELD, JOSEPH V. (See HOFFMAN AND MANSFIELD), 330
- MARCUS, ISRAEL H. Complete temporary recovery, of long duration in acute aleucemic myeloid leucemia, 1006
- MARTIN, DONALD S., AND SMITH, DAVID T. Laboratory diagnosis of blastomycosis, 1289
- MATTAS, CLYDE L. Adaptability of Lidberg paraffin embedding oven for various types of tissue work, 830

- MCCULLAGH, D. ROY, JAGLENSKI, T., KLOBU-CAR, F. Oxygen absorbing power in presence of certain diseases, 732
- MCEWEN, CURRIER, ALEXANDER, R. C., AND BUNIM, JOSEPH J. Bacteriologic and immunologic studies in arthritis, 453
- , BUNIM, JOSEPH J., AND ALEXANDER, R. C. Bacteriologic and immunologic studies in arthritis, 465
- MCLEAN, J. A round table for use in pathologic histology, 766
- MELMAN, MILDRED. (See MISHULOW, MELMAN, AND SKLARSKY), 406
- METZ, M. H. (See SIMON AND METZ), 1154
- MEYER, OVID O. (See KRUEGER AND MEYER), 682
- MILLER, DAVID. (See GALE AND MILLER), 31
- MILLER, ISIDORE. Blood sedimentation rates in middle-aged and old people, 1227
- MILLS, MOORE A. (See DRAGSTEDT AND MILLS), 1306
- MINOT, A. S., AND KELLER, MARGARET. A modification of Greenberg technic for colorimetric determination of serum protein, 743
- MIROLUBOVA, TATIANA N. (See GREENBERG AND MIROLUBOVA), 431
- MISHULOW, LUCY, MELMAN, MILDRED, AND SKLARSKY, RENA. Mouse protection test for standardizing antimeningococcus serums, 406
- MOLITCH, MATTHEW, AND COUSINS, RICHARD F. Subclinical scurvy in children, 43
- MORRISON, SAMUEL. The importance of the oxyntic cell in pernicious anemia, 828
- , GARDNER, RAYMOND E., AND REEVES, DAVID L. Selective elimination of neutral red through the gastric mucosa, 822
- MORTILLARO, MARY. (See HAZEN AND MORTILLARO), 710
- MULHERN, M. E., AND SEELYE, WALTER BALE. A case of meningitis in a newborn infant due to slow lactose-fermenting organism belonging to colon bacillus group, 793
- MURDOCK, THOMAS P. (See WELCH, MURDOCK, AND FERGUSON), 1264
- MYERS, JOHN A. A convenient and reliable instrument for electrical stimulation experiments, 949

N

- NFALE, R. C. (See FORBES, NEALE, HITE, ARMISTEAD, AND RUCKER), 1036
- NEELY, J. MARSHALL. The value of biopsy, 1124
- NEILD, H. W. (See BURGE, ORTH, NEILD, KROUSE, AND WICKWIRE), 1162
- NELSON, VICTOR E. (See KEIL AND NELSON), 1119
- NICE, L. B. (See KATZ AND NICE), 1145
- NICHOL, E. STERLING. Geographic distribution of rheumatic fever and rheumatic heart disease in the United States, 588
- NOONAN, W. J. (See HESSELTINE AND NOONAN), 281
- NORTON, JOHN F., DINGLE, JOHN H., AND SHENSTONE, T. HERBERT. A ball mill for grinding small quantities of bacteria, 1083

O

- O'REILLY, SISTER N. — of polymorphonuclear leukocytes in experimental infection of rat, 1137
- ORTH, O. S. (See BURGE, ORTH, NEILD, KROUSE, AND WICKWIRE), 1162
- OUTHIER, VIRGIL. (See BALLYEAT, SEYLER, AND OUTHIER), 187

P

- PIERCE, H. F. A metabolism chamber which automatically maintains a constant partial pressure of oxygen, 317

- PODOLSKY, EDWARD. Doctor as author, 32
- , Doctor as contributor to civilization, 169
- POSEN, EDNA. (See KOSTER, SHAPIRO, AND POSEN), 1096
- POWELL, H. M., AND JAMIESON, W. A. Preparation of Krueger undenatured bacterial antigens, 301
- PRICKETT, PAUL S., AND GARRETT, ORVILLE W. A rapid flushing-washing apparatus for laboratory glassware, 942
- PULVERTART, R. J. V. (See FRAENKEL AND PULVERTART), 359
- PUTNAM, TRACY. (See HYNDMAN AND PUTNAM), 850

R

- RADWIN, LEO S. AND LEDERER, MAX. Relation of bacterial infection to liver injury, 1047
- RAGINS, ALEX B. Pathogenesis of tuberculous leptomeningitis, 1217
- RAWLS, WILLIAM B., AND CHAPMAN, GEORGE H. Experimental arthritis in rabbits, 19
- REEVES, DAVID L. (See MORRISON, GARDNER, AND REEVES), 822
- RICE, CAROL M. Primary carcinoma of lung, 906
- RICHARDSON, A. P. (See LEHMAN, RICHARDSON, AND HANZLIK), 95
- RIGDON, R. H. The age of sexual maturity in 250 albino female rats, 1182
- RINEHART, JAMES F. An outline of studies relating to vitamin C deficiency in rheumatic fever, 597
- RINKEL, HERBERT J. Leucopenic index, 814
- ROBERTS, ELLA. (See GRIFFITH, ROBERTS, AND JEFFERS), 1208
- ROBERTSON, H. F. (See COX, HAWKINS, AND ROBERTSON), 192
- ROBINSON, W. L. An improved system of — s and slides, 1308
- TYLER, RICHARD. Serum containing heterophile antibody, 721
- ROWE, ALBERT H. (See GRAESER AND ROWE), 1131
- RUCKER, S. L. (See FORBES, NEALE, HITE, ARMISTEAD, AND RUCKER), 1036
- RYTZ, F. A rapid flocculation method for diagnosis of syphilis, 931

S

- SABINE, DAVID B. A comparison of media for plating *L. acidophilus*, 848
- SAHYUN, MELVILLE. Modification of Folin and Wu's method for sugar determination, 1089
- SAMUEL, EMMA LOUISE. (See KUGELMASS AND SAMUEL), 655
- SANFORD, HEYWORTH N. (See LESLIE AND SANFORD), 1078
- SANIGAR, EDWARD B., AND ALLEN, ALEXANDER J. A laboratory electric furnace suitable for micro-Kjeldahl digestions and similar uses, 969
- SAUNDERS, JOHN M. Action of magnesium in guanidine intoxication, 1236
- SCARF, MAXWELL. Gastrointestinal manifestations of hyperthyroidism, 1253
- SCHIEINBERG, DAVID. The response of blood urea nitrogen, uric acid and plasma cholesterol to parenteral liver extract, 690
- SCHLEICHER, EMIL MARO. Schilling's hemogram, 1296
- , (See SHARP AND SCHLEICHER), 975
- SCHMIDT, E. G., EASTLAND, J. S., AND BURNS, J. H. A comparative study of glucose and sucrose tolerance tests, 13
- , AND —. Influence on amino acid nitrogen and hemoglobin of blood, 1
- , AND —. Influence of sucrose ingestion on amino acid nitrogen and urea nitrogen concentration of blood, 233
- SCHMULOWITZ, M. J., AND WYLIE, H. BOYD. Chemical diagnosis of pregnancy by detection of estrin in urine, 210

- SCHUBB LURCH L. G. Blood sugar in uncomplicated and untreated neurosyphilis 401
- SCHULTZ CARL BRIANT (See HELWIG AND SCHULTZ) 261
- SCHWARTZ ISRAEL Blood calcium determination using standard calcium chloride solution 425
- SCOVERN CHARLES E. JR. Frozen stopcocks 317
- SCLODDER SARA A. Newfeld reaction in certain cases of pneumococcal septicaemia 168
- SEELIFF WALTER BILL (See MULLER AND SEELIFF) 793
- SEYLER L. EVERETT (See BAILLAT SEYLER AND OUTHIER) 187
- SHAPIRO ARTHUR (See KOSTER SHAPIRO AND POSEY) 1096
- SHAPIRO M. J. Nutritional history of childhood rheumatism in Minnesota 361
- SHARP E. A. AND SCHLICHTER, I. M. Bock's erythrocytonuclear unit technique for its use 915
- SHARPE JOHN C. AND BISBARD J. DWYER Relation of thyroid gland to hemiparesis 317
- SHAW FREDERICK W. An abridged key to species of pathogenic fungi 413
- SHELANSKI H. A. Studies on *T. vaginalis* in vitro 799
- SHEENSTONE T. HIRBLER (See NORTON DINGLE AND SHEENSTONE) 1033
- SHORT JAMES J. (See KELLEY AND SHORT) 910
- SIMON JOHN F. Effects of hypopycnea on human blood count blood chemistry and urine 409
- SIMON S. D. AND METZ M. H. Amidopyrine and circulating leucocytes 111
- SALARSKY RENA. (See MISHULOW MELMAN AND SALARSKY) 406
- SMITH DAVID T. (See MARTIN AND SMITH) 1239
- SMITH LAWRENCE W. (See BRODSKY SMITH AND WOLFF) 705
- SNIDER R. G. TRAEGER C. H. ZOIT C. L. KELLY LEMOYNE C. AND LIST F. J. The use of cinchophen in treatment of chronic arthritis 311
- SOLIS COHEN MYER Bacteriologic studies of urine utilizing a selective method of culturing 75
- SPOHR CARL L. AND LANDY MAURICE A cultural method for diagnosis of gonorrhea employing the direct oxidase reaction 69
- SPRENKEL VAUGHAN AND STEWART HAROLD L. Congenital thinning of wall of right anterior aortic sinus of aortal valve 128
- STELVER L. G. AND WOLFAW S. F. Diuretic action of metopurine 298
- STEVENS FRANKLIN A. The bactericidal action of irradiated oil of pine on hemolytic staphylococcus 1040
- The bactericidal and photochemical properties of irradiated petrolatum in mineral oil 26
- STEWART HAROLD L. (See SPRENKEL AND STEWART) 128
- STOVL WILLIAM S. Method of immunoblastocystis hominis from cultures of entamoeba histolytica 130
- Method of staining protozoa in bulk 839
- STROUD W. D. Discussion on paper by Dr. Hugh McCulloch. Institutional provisions for the care of the rheumatic child 618
- STRUMIA MAX M. A rapid universal blood stain 930
- STUART C. A. (See WILCH AND STUART) 411
- SUTTON DON C. (See LEITCH AND SUTTON) 1066
- SUTTON LUCY EDITH AND DICK KATHY ARNOLD C. Heat therapy in children in rheumatic conditions with and without fever 615
- SWAIN LORING T. What can be expected from orthopedic care of arthritis 33
- SWIFT MARTIN H. (See HEALY, DALLA, AND SWIFT) 635
- SWIFT HOMER F. Nature of rheumatic fever 331
- T
- TAMURA JOSEPH T. Rapid presumptive diagnosis of lymphogranuloma inguinale 817
- TAYLOR G. DOUGLAS FERGUSON A. B. KASABACH HAIG AND DAWSON M. H. A study of ionogenologic findings in various types of chronic arthritis 491
- THOMAS WILLIAM S. (See TOUART THOMAS AND TUCKER) 361
- TOIRRE JOHN C. AND LAKE MICHAEL Some observations on gastric acidity in relation to gastroduodenal and colonic disorders and an associated anemia 3170
- TOUART MAXIMIN D. THOMAS WILLIAM S. AND TUCKER WILLIAM L. A study of effects of vaccine injections upon skin sensitivity 365
- TOWNSEND ISABELLE M. AND COCA ARTHUR F. On some practical consequences of influence of temperature upon isohemagglutination 73
- TRAUGER C. H. (See SNIDER TRAEGER ZOLL KELLY AND LIST) 541
- TRAUT LUGENL F. (See CARTER AND TRAUT) 610
- TSUCHIDA H. The effects of dyes on endamoeba histolytica in vitro 1023
- TUCKER WILLIAM L. (See TOUART THOMAS AND TUCKER) 365
- TYLER RICHARD (See ROCKWELL AND TYLER) 721
- TYSON T. LLOYD (See DAWSON AND TYSON) 375
- U
- ULRICH HELMUTH A new diluting pipette for Sahli hemoglobinometer 705
- V
- VALENTINE ELINOR H. (See CROCKLER AND VALENTINE) 883
- VALENTINE LUCIENIA AND FALK R. GEORGE Fermentation and gas production by *B. coli* in simple and mixed sugars 27
- VAN DE ERVE J. (See KINARD AND VAN DE ERVE) 1203
- VAN LIEFER, D. J. A respiratory chamber for producing anoxemia in man 963
- VAN WINKLE CHARLOTTE C. (See WALD AND VAN WINKLE) 844
- VARNNEY PHILIP L. An automatically recording colony counting apparatus 297
- VARGHAN WARREN T. Automatic euthanasia 332 (C)
- Leucopenic index as a diagnostic method in study of food allergy 178
- Theory concerning the mechanism and the significance of allergic response 629
- Tissue penetration 109 (C)
- W
- WALD HUBERT AND VAN WINKLE CHARLOTTE C. A comparison of Ziehl-Neelsen and Spangler techniques of staining tubercle bacillus 844
- WALLBACH GILBERT Morphologic sugar metabolism in human leucocyte culture 163
- WALKER BURNHAM S. AND WALKER ELISABETH W. Normal magnesium metabolism and its significant disturbances 713
- WALKER ELISABETH W. (See WALKER BURNHAM S. AND WALKER ELISABETH W.) 713
- WALKER (POWELL) A. Satisfactory drinking fountain for caged animals 1087
- WALD MURIEL (See CRANE AND WALD) 1331
- WARRIN WIRT A. (See HADEN AND WARRIN) 118
- WALD RUSSELL A. An improved electro-polygraph 861

- WELCH, HENRY, AND STUART, C. A. A rapid slide test for serologic diagnosis of typhoid and paratyphoid fevers, 411
- , MURDOCK, THOMAS P., AND FERGUSON, JOHN A. Subacute bacterial endocarditis produced in rabbits with streptococci that resemble diphtheroids, 1261
- WERCH, S. CARL. The use of liquid air in cooling knives and of gelatin for mounting in frozen section technic, 1309
- WICKWIRE, G. C. (See BURGE, ORTH, NEILD, KROUSE, AND WICKWIRE), 1162
- WILLIAMS, JOHN E. (See MALTBY AND WILLIAMS), 354
- , Use of polar diagram in charting incidence of disease, 1303
- , AND GREEN, LEO. Weight loss of tubes of certain pathogenic fungi growing on a specific medium, 785
- WOHL, MICHAEL G., AND ETTELSON, L. N. Water retention in obesity as determined by Volhard dilution and McClure-Aldrich tests, 390
- WOLFF, WILLIAM I. (See BRODY, SMITH, AND WOLFF), 705
- WOLPAW, S. E. (See STEUER AND WOLPAW), 298
- WYLIE, H. BOYD. (See SCHMULOVITZ AND WYLIE), 210
- Y
- YOUNGBURG, GUY E., AND YOUNGBURG, MAMIE V. Phosphorus metabolism, 798
- YOUNGBURG, MAMIE V. (See YOUNGBURG, GUY E., AND YOUNGBURG, MAMIE V.), 798
- Z
- ZELLER, MICHAEL. The leucopenic index in vasomotor rhinitis, 1271
- ZOLL, C. A. (See SNYDER, TRAEGER, ZOLL, KELLY, AND LUST), 511

SUBJECT INDEX

Abstracts are indicated by (Abst) after the page number, book reviews, by (E. Rev.) after the page number

A

- Abscess, lung, 1076
 Absorbing power, oxygen, in presence of certain diseases, 732
 Abstracts, 99, 217, 323, 437, 659, 770 873, 983, 1093, 1211, 1310
 Acetylene method of determining cardiac output to dog, attempts to apply, 354
 Agglutinin response to typhoid vaccine, study of, 325 (Abst)
 Agranulocytic angina, 1213 (Abst)
 Air, liquid, use of, in cooling knives and of gelatin for mounting in frozen section technic, 1309
 Albino female rats, age of sexual maturity in 270 1152
 Albuminuria, functional, 221 (Abst)
 Alcaligenes group, microorganisms of, a hitherto undescribed, 710
 Aleuemic myeloid leucemia, acute, complete temporary recovery of long duration in, 1006
 Allergic dermatoses, 877 (Abst)
 diseases, intractable, concerning nature of food sensitization in, 811
 response, theory concerning mechanism and significance of 629
 Allergy and immunity, clinical study of, 659 (Abst)
 food, leucopenic index as a diagnostic method in study of, 1279
 Alum-diphtheria toxoid precipitate, chemical study of, 157
 Amebiasis, laboratory diagnosis of, sources of error in 1149
 American Association for the Study and Control of Rheumatic Diseases, 447-550 551 628
 Amidopyrine and allied drugs relation of, to etiology of agranulocytic angina, 1213 (Abst)
 and circulating leucocytes 1154
 Amino acid nitrogen, influence of dextrose ingestion on, urea nitrogen and hemoglobin concentration of blood, 1
 sucrose ingestion on and urea nitrogen concentration of blood, 231
 Amyloidosis Congo red test for, an improved, 93
 Anachility, gastric, some observations on, in relation to gastroduodenal and colonic floras and an associated anemia, 1170
 Anaerobic bacilli infections by gas-forming, 877 (Abst)
 Anaphylactic shock prevention of, due to horse serum by injection of BCG, 359
 Anatomy, morbid, postmortems and, 479 (E. Rev)
 Anemia, 659 (Abst)
 associated, some observations on gastric acidity in relation to gastroduodenal and colonic floras and an, 1170
 chronic hypochromic bile pigment and regeneration of effect of bile pigment in, 770 (Abst)
 experimental, absorption, storage, and elimination in, 1112 (Abst)
 iron and its utilization in 1713 (Abst)
 of premature infants 953 (Abst)
 of prematurity, 303 (Abst)
 pernicious, importance of oxyntic cell in, 524
 Angina pectoris experimental and clinical observations regarding, and some related symptoms, 993
 Animals, drinking fountain for caged, a satisfactory, 1057
 growth and space restriction, 655
 Annals of Pickett-Thomson laboratory, 108 (E. Rev)
 Anoxemia in man, respiratory chamber for producing, 963
 Anthrax, studies on, 134
 Antibody, heterophile, antipneumococcus serum containing, 721
 Antigen, Bordet and Ruelens, in complement fixation test for syphilis, a study of non-specific reactions of cerebrospinal fluids with, 1185
 -extracts, influence of composition of, for some serodiagnostic tests on syphilis (M B R 11 and M K R 11), 771
 Krueger undenatured bacterial, preparation of, 301
 rapid method for preparing, for Wassermann reaction, 417
 Antilymphogranuloma inguinale goat serum a specific intradermal test with, 842
 Antimeningococcus serums, mouse protection test for standardizing 106
 Antipneumococcus serum containing heterophile antibody, 721
 Aorta and bacterial endocarditis, slight distention of, 128
 Aortic sinus of Valsalva congenital thinning of wall of right anterior, 128
 Appendicitis, hemography in diagnosis of, based on 500 cases, 883
 in children, filament, nonfilament leucocyte count in, 875 (Abst)
 Arteriosclerosis 340
 Arthritis acute hypertrophic, accelerating factors in, 448
 atrophic (rheumatoid) and hypertrophic, protein studies in, 478
 treatment of, with leucocyte concentrate, 336
 bacteriologic and immunologic studies in, 453, 405
 blood cultures in different forms of, results of, 453
 chronic atrophic, effect of a high carbohydrate diet and insulin on symptoms and respiratory metabolism, 505
 cinchophen in treatment of, use of, 541
 diet in, studies on effect of a high-sulphur low-carbohydrate, 1036
 home treatment of, by physical therapy, 497
 infectious (atrophic), present status of fever therapy in treatment of gonorrheal arthritis and other forms of rheumatism 524
 roentgenologic findings in various types of, a study of 491
 constitution and, 1022
 experimental, in rabbits 49
 immunologic and bacteriologic studies 454, 465
 tests in different forms of, results of various 465
 orthopedic care of, what can be expected from 532
 -producing ability of magglutinable streptococci, comparison of, 49
 rheumatoid and rheumatic fever, relationship between 575
 treatment of chronic, 221 (Abst)
 synovial fluid in, characteristics of 1099 (Abst)
 Asbestosis, pulmonary, study of sputum in 217 (Abst)
 Aschoff body, 442 (Abst)

- Atrophic (rheumatoid) and hypertrophic arthritis, protein studies in, 178
 arthritis, chronic effect of a high carbohydrate diet and insulin on symptoms and respiratory metabolism, 505
 treatment of, with leucocyte concentrate, 536
 Auricular flutter with 1:1 response, 670
 Autohemagglutinin, 697
 Automatic euthanasia, 332 (C)
- B
- Bacilli, gas-forming anaerobic, infections by, 323 (Abst)
 tubercle, 325, 662 (Absts)
 and other microorganisms, mechanical device for preparing fine suspensions of, 428
Bacillus coli, fermentation and gas production by, in simple and mixed sugars, 257
 diphtheriae, rapid method for identification of, 411 (Abst)
 tuberculosis, synthetic medium for, 1215 (Abst)
 welchii toxin, intratracheal injections of, in rabbits, evanescent effect of, 659 (Abst)
 tubercle, 1311 (Abst)
 progeny of, 1101 (Abst)
 staining comparison of Ziehl-Neelsen and Spengler techniques of, 544
 Bacteria, ball mill for grinding small quantities of, 1083
 Bacterial antigens, Krueger undenatured, preparation of, 301
 endocarditis, slight dextroposition of aorta and, 125
 subacute, produced in rabbits with streptococci that resemble diphtheroids, 1261
 flora associated with foreign bodies in trachea and bronchi, 326 (Abst)
 infection, relation of, to liver injury, 1047
 Bactericidal action of irradiated oil of pine on hemolytic streptococcus, 1040
 of short and ultrashort waves, 31
 and photochemical properties of irradiated petrolatum and mineral oil, 26
 Bacteriemia in pneumonia, incidence of, 323 (Abst)
 Bacteriologic and immunologic studies in arthritis, 433, 465
 studies on fecal streptococci and lactic acid streptococci, 111
 of urine, utilizing a selective method of culturing, 775
 Bacteriology, 774 (Abst)
 experimental, 882 (B Rev)
 textbook of, 224 (B Rev)
 general, 223 (B Rev)
 Banti's disease, relation of chronic congestive splenomegaly to, 217 (Abst)
 Basal and exercise cardiac output on dogs, method of estimating both, 192
 oxygen consumption, expiratory chest volume during routine measurement of, frequency and significance of changes of, 1231
 Basophilia of granules of polymorphonuclear neutrophilic leucocytes, production of, in experimental infection of rat, 1137
 Bisophilic aggregation counts fluctuations in, with meteorologic alterations, 677
 BCG, anaphylactic shock due to horse serum by injection of, prevention of, 359
 Beverage and food analyses, 443 (B Rev)
 Bichloride poisoning treatment of, 660 (Abst)
 Bile pigment and regeneration of effect of bile pigment in chronic hypochromic anemia, 1770 (Abst)
 Biopsy, value of, 1124
 Bismuth in body fluids and tissues, improved procedures for estimating, 95
 Buret method, determination of fibrin by, 1084
 Bladder, urinary, tumors of 444 (B Rev)
 Blastocystis hominis, method of eliminating, from cultures of entamoeba histolytica, 190
 Blastomycosis, laboratory diagnosis of, 1259
 Blood agar cultures and Dick test as aids in diagnosis of scarlet fever, 875 (Abst)
 amino acid nitrogen and urea nitrogen concentration of, influence of sucrose ingestion on, 233
 calcium, 986 (Abst)
 determination, using standard calcium chloride solution, 425
 cells, white, phospholipid content of, estimation of, 957
 effect of prolonged administration on number and type of, 1154
 chemistry, effects of hyperpyrexia on human blood count, urine and, 100
 clotting and citrated, observations on pH of, 663
 count, blood chemistry and urine, effects of hyperpyrexia on human, 400
 cultures in different forms of arthritis, results of, 453
 diseases, clinical atlas of, 223 (B Rev)
 puncture of spleen in, 1312 (Abst)
 disorders of, 222 (B Rev)
 fat determination, total, as an index of thyroid function, 1310 (Abst)
 glucose clearance, 986 (Abst)
 grouping in infectious diseases, 705
 in healthy young infants, 772, 773 (Absts)
 in spinal fluid, a staining technique for, 1205
 hemoglobin concentration of, influence of dextrose ingestion on amino acid nitrogen, urea nitrogen and, 1
 peripheral, in lobar pneumonia, 659 (Abst)
 picture in experimental whooping cough, 99 (Abst)
 phosphorus, changes and relationships in, of rats subjected to blood regeneration by repeated bleedings, 798
 platelets, effect of obstructive jaundice on, of rabbit, 899
 pressure, capillary, cutaneous, new method for determination of, 179
 sedimentation, a new method and a new pipette for, 971
 rate in diabetes mellitus 37
 in middle-aged and old people, 1227
 serum phosphomolybdic acid centrifugates, calcium in, effect of hydrogen ion concentration upon determination of, 65
 spinal fluid contaminated by, diagnosis in, 100 (Abst)
 stain, polychrome, a general purpose, 978
 a rapid universal, 930
 sugar level after prolonged carbohydrate feeding, 1142
 in uncomplicated and untreated neurosyphilis, 404
 for tissue culture experiments, a technique for obtaining human 940
 transfusion, transmission of syphilis by, factors conditioning, 877 (Abst)
 urea in nephritic rats, comparison of xylene tolerance with, 1010
 nitrogen, response of, uric acid and plasma cholesterol to parenteral liver extract, 690
 Bock's erythrocytometer and technique for its use, 975
 Body fluids and tissues bismuth in improved procedures for estimating, 95
 Bone marrow, neoplastic reaction of, to liver extract, 335
 Book reviews 104, 222, 443, 879
 Bordet and Ruelens antigen in complement fixation test for syphilis, a study of nonspecific reactions of cerebrospinal fluids with, 1185
 Box, mouse, for operating on the tail, 1302
 Breast lesions, 219 (Abst)
 tumors, 874 (Abst)
 Bright's disease, significance of serum inorganic sulphate concentrations in, 380
 Bromsulphalein dye retention test employment of oxalated plasma in, 1306
 Bronchiectasis, 220 (Abst)

C

- Calcium blood J86 (Abst.)
determination using standard calcium chloride solution 12
in blood serum phosphomolybdic cell centri-
fugate effect of hydrogen ion
concentration upon determination
of 65
chloride solution blood calcium determina-
tion using standard 42
neutralize thyroxine does 1131
Cancer 1314 (Abst.)
Roffo's test in 2.1 (Abst.)
Capillary blood pressure new method for de-
termination of cutaneous 179
resistance test application of 43
Carbohydrate diet and insulin effect of high
on symptoms and respiratory me-
tabolism chronic atrophic ath-
ritis 69
feeding blood sugar level after prolonged
1142
Carbon dioxide simple method of supplying
in jars for bacteriologic cultures
774 (Abst.)
Carcinoma early cutaneous 101 (Abst.)
primary of lung 908
of stomach diagnosis of from a fragment
of tumor obtained during routine
gastric analysis 836
Cardiac output to dog attempts to apply
acetylene method of determining
364
basal and exercise method of estimating
both 192
Cardiology recent advances in 881 (B Rev.)
Carditis rheumatic with and without chorea
fever therapy in chorea and in
619
Casting and molding for medical sciences
technic of 68
Cell sickle 873 (Abst.)
white blood phospholipid content of cell
matter of 967
Cerebrospinal fluid 1311 (Abst.)
during and between attacks of migraine
headaches 327 (Abst.)
procaine in a method for microdetermina-
tion of 1096
differentiation of 1215 (Abst.)
nonspecific reactions of a study of with
Bordet and Ruelens antigen in
complement fixation test for syph-
ilis 1186
Cinchophen intoxication studies in 1047
in treatment of chronic arthritis use of
641
Circulation failure of 104 (B Rev.)
Citrate sodium—a spirocheticide 922
Civilization doctor as contributor to 169
Chemical diagnosis of pregnancy by detection
of estrin in urine 210
study of alum diptheria toxoid precipitate
167
test for pregnancy 986 (Abst.)
Chemistry blood effects of hyperpyrexia on
human blood count, urine and 400
Chenotaxis comparative studies in 1017
Chest volume expiratory frequency and sig-
nificance of changes of during
routine measurement of basal oxy-
gen consumption 1231
Childhood rheumatism natural history of in
Minnesota 564
Cholesterol plasma response of blood urea
nitrogen urea cell and to pan-
creatic liver extract 690
studies of plasma proteins and 340
Chorea fever therapy in and in rheumatic
arthritis with and without chorea
619
Clock a synchronous motor electric time
57
Colon bacillus group slow lactose fermenting
organism belonging to a case of
meningitis in a newborn infant due
to 793
Colony counting apparatus an automatically
recording 207
Color photography three a method of 860

- Colorimetric analyses limitations of by pres-
ent methods 181
determination modifications in. of plasma
proteins by 101m phenol reagent
431
of serum protein a modification of Green-
berg technic for 743
Complement fixation reactions influence of
physiologic salines in 1063
test for gonorrhea a more sensitive 308
for syphilis a study of non-specific reac-
tions of cerebrospinal fluids with
Bordet and Ruelens antigen in
1186
titrations inadequacy of present 312
Congenital syphilis 773 (Abst.)
thinning of wall of right anterior aortic sinus
of vulva 128
Congo red test for amyloidosis an improved
93
Constitution and arthritis, 1022
Copper and iron in metabolism further studies
on 111
Corpuscles sheep prolonged preservation of
antigenic specificity of associated
with production of marked increase
in resistance to hypotonic solu-
tions 943
Correspondence 992 1216 1314
Counts nonfilament and filament, relation of
during excitement 1145
Counting apparatus an automatically record-
ing colony 207
Creatinuria of infancy and childhood 1310
(Abst.)
Criminal investigation modern 882 (B Rev.)
Cryptococci and monilia, culture and mor-
phologic studies of isolated from
vulvovaginitis and oral thrush
1113
Cultural method for diagnosis of gonorrhea
employing direct oxydase reaction
650
and morphologic studies of cryptococci and
monilia isolated from vulvovagi-
nitis and oral thrush 1113
Cap food for rats a convenient 1194
Cytology of pleural effusion studied with a
supravital technic 659 (Abst.)

D

- Dental periapical infections red blood cell
sedimentation rate in chronic sin-
usitis chronic tonsillitis and 1259
Dermatoses allergic 87 (Abst.)
Device for drying pipettes and other labora-
tory glassware a simple efficient
and inexpensive 435
Dextrose content of lumbar and cisternal cere-
brospinal fluid 1311 (Abst.)
Ingestion of amino acid nitrogen influence
of urea nitrogen and hemoglobin
concentration of blood 1
tolerance 1213 (Abst.)
Diabetes mellitus blood sedimentation rate in
36
treatment of evaluation of hyperglycemia in,
438 (Abst.)
Diabetic individual safely induced fever ther-
apy in a 860
Diagram polar in charting incidence of dis-
ease use of 1303
Dick test and blood agar cultures as aids in
diagnosis of scarlet fever 87,
(Abst.)
Diet high sulphur low carbohydrate studies
on effect of in chronic arthritis
1036
Diphtheria 441 1101 (Absts.)
Diphtheroids streptococci that resemble sub-
acute bacterial endocarditis pro-
duced in rabbits with 1264
Disease use of polar diagram in charting in-
cidence of 1303
Diuretic action of mercaptopurine 298
Doctors as author 32
as contributor to civilization 169
woman asks 108 (B Rev.)
Doctors and juries 223 (B Rev.)
for and against 881 (B Rev.)

- Drinking fountain for caged animals, a satisfactory, 1087
 Dyes, effects of, on endameba histolytica in vitro, 1028
 Dye retention test, bromsulphalein, employment of ovalated plasma in, 1306

E

- Eagle, Kolmer-Wassermann, and Kahn precipitation tests, a study of relative sensitivity and specificity of, 952
 Editorial, automatic euthanasia, 332
 organization in study and control of rheumatic diseases, 518
 renal function tests, 989
 thoughts on the education of the public, 330
 tissue penetration, 109
 Education of the public, thoughts on the, 330 (E)
 Electric furnace, laboratory, suitable for micro-Kjeldahl digestions and similar uses, 969
 Electrical stimulation experiments, a convenient and reliable instrument for, 949
 Electronegativity, cause and significance of, of active living tissue, 1162
 Electropolygraph, an improved, 861
 Empyema, putrid, 219 (Abst.)
 Encephalitis, acute, 661 (Abst.)
 fulminating hemorrhagic, 875 (Abst.)
 Encephalopathy, lead, simulation of intracranial tumor, 325 (Abst.)
 Endameba histolytica in vitro, effects of dyes on, 1028
 Endocarditis, bacterial, slight dectroposition of aorta and, 128
 subacute bacterial, produced in rabbits with streptococci that resemble diphtheroids, 1261
 Endocrine diagnosis and treatment, recent advances in, 736
 Entamoeba histolytica, cultures of, method of eliminating blastocystis hominis from, 190
 Eosinophiles in rheumatic fever, behavior of, 225
 Eosinophilia in scarlet fever, 323, 878 (Absts.)
 Epinephrine by inhalation, administration of, 1134
 Erythrocyte permeability, methods for determining, 308
 Erythrocytes in salt solution, a method for measuring fragility of, 833
 Erythrocytometer, Bock's, and technic for its use, 975
 Experiments, the design of 144 (B Rev.)
 Estrin in urine, chemical diagnosis of pregnancy by detection of, 210
 Euthanasia, automatic, 332 (E)
 Excitement, nonfilament and filament counts during, relation of, 1145

F

- Fecal streptococci and lactic acid streptococci bacteriologic studies on, 111
 Fermentation and gas production by *B. coli* in simple and mixed sugars, 257
 tests, reliability of in identification of monilias, 1105
 Fever, rheumatic, and rheumatoid arthritis, relationship between, 575
 behavior of eosinophiles in, 225
 geographic distribution of, and rheumatic heart disease in United States, 588
 nature of, 551
 vitamin C deficiency in an outline of studies relating to 597
 scarlet, 875 (Abst.)
 therapy in chorea and in rheumatic carditis with and without chorea, 619
 safely induced, in a diabetic individual, 820
 in treatment of gonorrheal arthritis, chronic infectious (atrophic) arthritis, and other forms of rheumatism, present status of, 524
 Fibrin, determination of, by Biuret method, 1084
 Flocculation method, rapid, for diagnosis of syphilis, 934

- Floras, colonic, some observations on gastric acidity in relation to gastroduodenal and, an associated anemia, 1170
 Fluctuations in basophilic aggregation counts with meteorologic alterations, 677
 Flushing-washing apparatus, rapid, for laboratory glassware, 942
 Flutter, auricular, with 1:1 response, 670
 Folin and Wu's method for sugar determination, modification of, 1089
 phenol reagent, modifications in colorimetric determination of plasma proteins by, 431
 Food allergy, leucopenic index as a diagnostic method in study of, 1278
 Food and beverage analyses, 443 (B Rev.)
 cup for rats, a convenient, 1194
 sensitization in intractable allergic diseases, concerning nature of, 814
 Foot, 879 (B Rev.)
 Fountain, drinking, for caged animals, a satisfactory, 1087
 Frozen section technic, use of liquid air in cooling knives and of gelatin for mounting in, 1309
 stopcocks, 847
 Fructosuria, essential, 770 (Abst.)
 Fungi, pathogenic, an abridged key to species of, 213
 weight loss of tubes of certain, growing on a specific medium, 785
 Fungicides, 281, 288
 Furnace, laboratory electric, suitable for micro-Kjeldahl digestions and similar uses, 969

G

- Gas production and fermentation by *B. coli* in simple and mixed sugars, 257
 Gastric acidity, some observations on, in relation to gastroduodenal and colonic floras and an associated anemia, 1170
 analysis, routine, diagnosis of carcinoma of stomach from a fragment of tumor obtained during, 836
 juice, concentrated human, hematopoietic material in, 220 (Abst.)
 mucosa, selective elimination of neutral red through, 822
 Gastroduodenal and colonic floras and an associated anemia, some observations on gastric acidity in relation to 1170
 Gastrointestinal manifestations of hyperthyroidism, 1253
 Geographic distribution of rheumatic fever and rheumatic heart disease in United States, 588
 Gingivitis and periodontoclasia, medical aspects of, 698
 Gland, thyroid, relation of, to hematopoiesis, 347
 Glassware, laboratory, a rapid flushing-washing apparatus for, 942
 Glomerular efficiency, sodium ferrocyanide as a clinical test for, 102 (Abst.)
 Glucose and sucrose tolerance tests, a comparative study of, 13
 blood, clearance, 986 (Abst.)
 Glycerol-free medium for tubercle bacillus, 1311 (Abst.)
 Glycosuria, 329 (Abst.)
 nondiabetic, incidence of, 152
 Goat serum, antilymphogranuloma inguinale, a specific intradermal test with, 842
 Gonococcus, 219 (Abst.)
 Gonorrhea, complement fixation test for, a more sensitive, 308
 cultural method for diagnosis of, employing direct oxydase reaction, 650
 in male, criteria of cure of, 219 (Abst.)
 Gonorrheal arthritis, fever therapy in treatment of, present status of, chronic infectious (atrophic) arthritis and other forms of rheumatism, 524
 Gout, preservation and microscopic examination of nodules in, 985 (Abst.)

- Granules basophilic of of polymorphonuclear neutrophilic leukocytes production of in experimental infection of rat 1137
- Greenberg technique a modification of for colorimetric determination of serum protein 743
- Gross specimens, pathologic library of, 9-6
- Grouping blood in infectious diseases, 703
- Guanine intoxication action of magnesium in 1236

H

- H and O agglutination of *B. typhosus* 662 (Abst.)
- Hair and scalp 879 (B Rev.)
- Harvard kymographs muscle nerve stimulating accessory for 869
- Harvey lectures 103 (B Rev.)
- Health organization quarterly bulletin of League of Nations 105 (B Rev.)
- Heart disease rheumatic geographic distribution of rheumatic fever and in United States 588
- Hematologic nomenclature 173
- Hematopoiesis relation of thyroid gland to 347
- Hematopoietic function in rabbit modification of by certain cyclic compounds 913
- material in concentrated human gastric juice, 220 (Abst.)
- Hemoglobin 770 (Abst.)
- concentration of blood influence of dextrose ingestion on amino acid nitrogen urea nitrogen and 1
- values in normal adults over a period of time 787
- Hemoglobinometer Sahli a new diluting pipette for 753
- Hemogram Schilling's 1296
- Hemography in diagnosis of appendicitis based on 300 cases 883
- Hemolytic streptococcus bactericidal action of irradiated oil of pine on 1040
- Hemophilia histopathology of hematopoietic tissues in 323 (Abst.)
- Hemorrhagic encephalitis fulminating 875 (Abst.)
- nephritis infection and, 440 (Abst.)
- Heterophile antibody antipneumococcus serum containing 721
- test in leucemia 878 (Abst.)
- High sulphur low carbohydrate diet in chronic arthritis studies on effect of 1036
- Histology pathologic a round table for use in 766
- textbook of 103 (B Rev.)
- Holkins disease 682
- (iology of 770 (Abst.)
- Home treatment of chronic arthritis by physical therapy 497
- Human blood for tissue culture experiments a technique for obtaining 940
- Hydrogen ion concentration effect of upon determination of calcium in blood serum phosphomolybdic acid centrifugates 65
- influence of on growth of yeastlike organisms 281
- Hyperglycemia in treatment of diabetes evaluation of 438 (Abst.)
- Hyperproteinemia multiple myeloma with 327 (Abst.)
- Hypopyrexia effects of on human blood count blood chemistry and urine 400
- Hypertension on chronic of nervous origin 235
- Hyperthyroidism gastrointestinal manifestations 1253
- quinine test for 123
- Hypertrophic and atrophic (rheumatoid) arthritis protein studies in 478
- arthritis (osteoarthritis) chronic accelerating factors in 448
- Hypoglycemia in newborn 429 (Abst.)
- Hypotonic solutions resistance to prolonged preservation of antigenic specificity of sheep corpuscles associated with production of marked increase in 943

I

- Ile test—new color test for syphilis 1130
- Illitis fulminant pneumonia with associated fatal case of 117
- Illumination dark field in diagnosis of tuberculosis and malaria 421
- Illuminator intestinal 223
- Immunity in allergy clinical study of 659 (Abst.)
- Immunologic and bacteriologic studies in arthritis, 453 460
- tests in different forms of arthritis results of various 463
- Impetigo contagiosa treatment for, 1214 (Abst.)
- Inaex leucopenic 811
- as diagnostic method in study of food allergy 1278
- in vasomotor rhinitis 1274
- opsonic modification of Wright's technique for determination of 1203
- Infants blood in healthy young 772 773 (Abst.)
- premature anemia of 983 (Abst.)
- Infection and hemorrhagic nephritis 440 (Abst.)
- bacterial relation of to liver injury 1047
- by gas forming anaerobic bacilli 323 (Abst.)
- rheumatic in children influence of tonsils on 609
- Infectious diseases blood grouping in 703
- Inhalation epinephrine by administration, 1134
- Insulin and high carbohydrate diet effect of on symptoms and respiratory metabolism 505
- intranasal application of 278
- Instrument for electrical stimulation experiments a convenient and reliable 949
- International Medical Annual 107 (B Rev.)
- Intestinal flora in newborn infants 1103 (Abst.)
- Illuminator 923
- Intoxication cinchophen studies in 1047
- guanidine action of magnesium in 1236
- Intradermal test a specific with antilymphogranuloma inguinale goat serum 842
- Intranasal application of insulin 278
- Introductory remarks 445
- Iolized oil 187
- Iron and copper in metabolism further studies on 1119
- and its utilization in experimental anemia 1313 (Abst.)
- Irradiated oil of pine bactericidal action of on hemolytic streptococcus 1040
- Isoagglutination influence of temperature upon on some practical consequences of 729

J

- Jaundice 1102 (Abst.)
- mechanism of 1100 (Abst.)
- obstructive effect of on blood platelets of rabbit 899
- study of 100 cases of with particular reference to galactose tolerance 217 (Abst.)

K

- Kahn and Wassermann reactions alleged variabilities of from meteorologic changes 910
- Kolmer Wassermann and Eagle precipitation tests a study of relative sensitivity and specificity of 952
- Kidney in health and disease 107 (B Rev.)
- Kolmer Wassermann Kahn and Eagle precipitation tests a study of relative sensitivity and specificity of 952
- Kuegel undenatured bacterial antigens preparation of 301
- Kymographs Harvard muscle nerve stimulating accessory for 869

L

- L. acidophilus*, media for plating, a comparison of, 818
- Laboratory diagnosis, 101 (B. Rev.)
- of amblyosis, sources of error in, 1119
- of blastomycosis, 1289
- dogs, control of mange in, 1203
- electric furnace suitable for micro-Kjeldahl digestions and similar uses, 969
- glassware, a rapid flushing-washing apparatus for, 942
- manual, 107 (B. Rev.)
- methods, clinical diagnosis by, 222 (B. Rev.)
- Pickett-Thomson, annals of, 108 (B. Rev.)
- timing device, an inexpensive, 860
- Lactic acid streptococci, bacteriologic studies on fecal streptococci and, 111
- Lactose-fermenting organism, a case of meningitis in a newborn infant due to a slow, belonging to colon bacillus group, 793
- Lead encephalopathy, 325 (Abst.)
- Leprosy, Mitsuda's skin reaction in, 771 (Abst.)
- Leptomeningitis, tuberculous, pathogenesis of, 1217
- Leucemia, acute aleucemic myeloid, complete temporary recovery of long duration in, 1006
- diagnosis and treatment of, 984 (Abst.)
- differential diagnosis of, 981 (Abst.)
- heterophile antibody test in, 878 (Abst.)
- Leucocyte, amidopyrine and circulating, 1151
- concentrate, treatment of atrophic (rheumatoid) arthritis with, 536
- count during artificial pneumothorax treatment and lung expansion, 660 (Abst.)
- in phagocytic tests, importance of, 760
- normal range of, determined weekly over an extended period, 376
- culture, human, morphologic sugar metabolism in, 163
- filament, nonfilament count in appendicitis in children, 875 (Abst.)
- polymorphonuclei neutrophilic, production of basophilia of granules of, in experimental infection of rat, 1137
- reaction in tuberculosis, 329 (Abst.)
- Leucopenic index, 814
- as diagnostic method in study of food allergy 1278
- in vasomotor rhinitis, 1274
- Library, pathologic, of gross specimens, 926
- Lidberg paraffin embedding oven for various types of tissue work, adaptability of, 830
- Liquid an in cooling knives and of gelatin for mounting in frozen section technique use of, 1309
- Liver extract, parenteral, response of blood urea nitrogen, uric acid and plasma cholesterol to, 690
- reaction of normoblastic bone marrow to, 335
- injury, relation of bacterial infection to 1047
- kidney syndrome, further contribution to 261
- therapy, parenteral, in streptococcus pneumonia, 988 (Abst.)
- treatment, lack of effect of, on circulating reticulocytes in picon, 660 (Abst.)
- Lobar pneumonia, 659 (Abst.)
- Low-carbohydrate high-sulphur diet in chronic arthritis, studies on (effect of, 1036
- Lung abscess, 1056
- primary carcinoma of, 906
- Lymphogranuloma inguinale, 439, 771 (Abst.)
- cultivation of virus of, 771 (Abst.)
- rapid presumptive diagnosis of, 842
- Lymphogranulomatosis (Hodgkin's disease) 682
- Lymphobathia venereum its relation to rectal structure, 328 (Abst.)
- Malara and tuberculosis, dark-field illumination in diagnosis of, 121
- Mange, control of, in laboratory dogs, 1203
- Maturity, sexual, age of, in 250 albino female rats, (*Mus norvegicus albus*, Wistar strain), 1182
- May-Gruenwald-Giemsa in one solution, 930
- Mechanical device for preparing fine suspensions of tubercle bacilli and other microorganisms, 128
- Mechanism and significance of allergic response, theory concerning, 623
- Media for plating *L. acidophilus*, comparison of, 818
- Medical aspects of periodontoclasia and gingivitis, 698
- sciences, technic of molding and casting for, 68
- Medicine in Middle Ages, story of, 106 (B. Rev.)
- tropical, and clinical parasitology, 413 (B. Rev.)
- Meningitis, 437 (Abst.)
- acute aseptic, 141 (Abst.)
- in newborn infant due to a slow lactose-fermenting organism belonging to colon bacillus group, a case of, 793
- tuberculous, 871 (Abst.)
- Mercurium, diuretic action of, 298
- Mercurochrome, 110 (Abst.)
- Metabolism chamber which automatically maintains a constant partial pressure of oxygen, 317
- copper and iron in further studies on, 1119
- magnesium, normal, and its significant disturbances, 713
- morphologic sugar, in human leucocyte culture, 163
- phosphorus, 798
- respiratory, effect of high carbohydrate diet and insulin on symptoms and, 505
- Meteorologic alterations, fluctuations in basophilic aggregation counts with, 677
- changes, alleged variabilities of Kahn and Wassermann reactions from, 910
- Microdetermination of procaine in cerebrospinal fluid, a method for, 1096
- of sodium by uranyl zinc acetate method and titration of uranium with cadmium as reductant, 1198
- Micro-Kjeldahl digestions, a laboratory electric furnace suitable for, and similar uses, 969
- Microorganism of alcaligenes group, a hitherto undescribed, 710
- Microreaction for serodiagnosis of syphilis, a new, 1201
- Migraine headaches, cerebrospinal fluid during and between attacks of, 327 (Abst.)
- Mill, ball, for grinding small quantities of, 1083
- Mineral oil and irradiated petrolatum, bactericidal and photochemical properties of, 26
- Molding and casting for medical sciences, technic of, 68
- Monilia and cryptococci, cultural and morphologic studies of, isolated from vulvovaginitis and oral thrush, 1113
- identification of, reliability of fermentation tests in, 1105
- Mononucleosis, infectious, 139, 1211 (Absts.)
- Mouse box for operating on the tail, 1302
- protection test for standardizing antilymphococcus serum, 406
- Mucosa, gastric, selective elimination of neutral red through, 822
- Muscle nerve stimulating accessory for Harvard kymographs, 869
- Myeloid leucemia acute aleucemic, complete temporary recovery of long duration in 1006
- Myeloma, Bence-Jones proteinemia in multiple, 984 (Abst.)
- multiple, with hyperproteinemia 327 (Abst.)
- Myocardial, studies on 442 (Abst.)

M

- Neck malignant epithelial tumors of 98
(Abst)
- Nephritic rate blood urea in comparison of
xylose tolerance with 1010
- Nephritis hemorrhagic infection and 440
(Abst)
- Nerve muscle stimulating accessory for
Harvard kymographs 869
- Nervous origin on chronic hypertension of
33
- Neufeld reaction in certain cases of pneumo-
coccal septicemia 168
- Neurosyphilis blood sugar in uncomplicated
d in
- N rough
- N
- N test
- N urini
- 119)
- Nitrogen blood urea response of urine cell
and plasma cholesterol to paren-
teral liver extract 690
- Normoblastic bone marrow reaction of to
liver extract 33
- O
- Obesity water retention in as determined by
Volhard dilution and McClure Al-
drich tests 390
- Oil iodized 40
of pine 40
- Opsonic ind 40
each
- Oral thrush and
morphologic studies of cryptococci
and monilia isolated from 1113
- Organization in study and control of intra-
matic diseases 318 (I)
- Oithopadic care of arthritis what can be ex-
pected from 532
- Osteoarthritis 448
- Oven Luberg paraffin embedding for various
types of tissue work adaptability
of 830
- Oxalated plasma in bromsulphalein dye test
nation test employment of 1306
- Oxydase reaction direct cultural method for
diagnosis of gonorrhea employ n
650
- Oxygen absorbing power in presence of certain
diseases 732
- consumption basal expiratory chest volume
during routine measurement of
frequency and significance of
changes of 1231
- metabolism chamber which automatically
maintains a constant partial pres-
sure of 31
- Oxyntic cell in pernicious anemia importance
of 828
- P
- Pain and weather 1247
- Paraffin embedding oven Luberg for vari-
ous types of tissue work adapt-
ability of 830
- Parasitology clinical and tropical medicine
443 (B Rev)
- Parityphoid and typhoid fevers a rapid slide
test for serologic diagnosis of 411
- Parenteral liver extract response of blood
urea nitrogen uric acid and
plasma cholesterol to 690
- Pathogenesis of tuberculous leptomeningitis
1217
- Pathogenic fungi in abridged key to species
of 243
- weight loss of tubes of certain growing
on a specific medium 78
- Peptic ulcer gastric mucin treatment of 98
(Abst)
- Periapical infections dental and blood cell
sedimentation rate in chronic si-
nusitis chronic tonsillitis and 129
- Periodontitis and gingivitis medical
aspects of 698
- Pernicious anemia importance of oxyntic cell
in 828
- Petrolatum irradiated and mineral oil, bac-
tericidal and photochemical prop-
erties of 26
- pH of clotting and clotting blood observa-
tions on 663
- Phagocytic tests importance of leucocyte
counts in 760
- Phenol reagent Folin modifications in colori-
metric determination of plasma
proteins by 431
- Phospholipid content of white blood cells
estimation of 957
- Phosphorus blood changes and relationships
in of rats subjected to blood re-
generation by repeated bleedings
798
- metabolism 798
- Photography three color method of 850
- Phthalum test for renal function 1103 (Abst)
- Physical therapy home treatment of chronic
arthritis by 497
- Pickett-Thomson Laboratory annals of 108
(B Rev)
- Pipette and other laboratory glassware a sim-
ple efficient and inexpensive de-
vice for diving 435
- for blood sedimentation a new method and
a new 371
- new diluting for Sahli hemoglobinometer
755
- Plague rodent in California 326 (Abst)
- Plasma cholesterol response of blood urea
nitrogen uric acid and to paren-
teral liver extract 690
- estimation of platelets in their own meth-
od of quantitative and qualitative
1078
- oxalated
- proteins
colorimetric
- tions in by Folin phenol reagent
431
- Platelets blood effect of obstructive junction
on of rabbit 899
- method of quantitative and qualitative esti-
mation of in their own plasma
1078
- Pneumococcal septicemia Neufeld reaction in
certain cases of 118
- Pneumococcus and streptococcus vaccines 218
(Abst)
- sodium desoxycholate for identification of
218 (Abst)
- Pneumonia 659 1215 (Absts)
- incidence of bacteremia in 323 (Abst)
- lobar 659 (Abst)
- streptococcus 988 (Abst)
- tubercular fatal case of with associated
ileitis 117
- Pneumothorax treatment artificial and lung
expansion leucocyte count during
660 (Abst)
- Poisoning bichloride treatment of 660
(Abst)
- Polar diagram in charting incidence of dis-
ease use of 1303
- Polymyositis 770 (Abst)
- vaccination against successful method for
100 (Abst)
- Polychrome blood stain a general purpose 978
- Polymorphonuclear neutrophilic leucocytes
production of basophilia of gran-
ules of in experimental infection
of rat 113
- Post-mortem and morbid anatomy 879 (B
Rev)
- Precipitation tests Kolmer Wassermann
Kahn and Eagle a study of rela-
tive sensitivity and specificity of
952
- Pregnancy and syphilis 983 (Abst)
- chemical diagnosis of by detection of estrin
in urine 10
- test for 986 (Abst)
- Prematurity anemia of 103 (Abst)
- Pressure thoracic duct lymph in constrictio
cordis 296
- Procaine in cerebrospinal fluid a method for
microdetermination of 1096

Protein, plasma, and cholesterol, studies of, 310
 colorimetric determination of, modifications in, by Folin phenol reagent, 131
 serum, colorimetric determination of, a modification of Greenberg technic for, 743
 determination, a rapid method for routine, 1092
 studies in atrophic (rheumatoid) and hypetrophic arthritis, 478
 Proteinemia, Bence-Jones, in multiple myeloma, 984 (Abst)
 Proteus X-19, 1104 (Abst)
 Protozoa, method of staining, in bulk, 833
 staining technic for, 1214 (Abst)
 Psoriasis, treatment for, simple, 1147
 Pulmonary asbestosis, study of sputum in, 217 (Abst)
 blood flow in silicosis, clinical observations on, 1313 (Abst)
 Pyuria, 438 (Abst)

Q

Quantitative and qualitative estimation of platelets in their own plasma, a method of, 1078
 method, rapid, for examining the urine in renal disorders, 98 (Abst)
 Quarterly Bulletin of Health Organization, League of Nations, 105 (B Rev)
 Quinme test for hyperthyroidism, 123

R

Rabies, 1213 (Abst)
 Reactions, complement fixation, influence of physiologic salines in, 1069
 tuberculin, 1101 (Abst)
 Wassermann, a rapid method for preparing antigens for, 117
 Rectal stricture, relation of lymphopithis venereum to, 328 (Abst)
 Red blood cell sedimentation rate in chronic sinusitis, chronic tonsillitis, and dental periapical infections, 1259
 Renal disorders, urine in, rapid quantitative method for examining, 98 (Abst)
 function, 102, 1099 (Absts)
 phthalein test for, 1103 (Abst)
 tests, 989 (D)
 tuberculosis, 1102 (Abst)
 Respiratory chamber for producing anoxemia in man, 963
 metabolism, effect of high carbohydrate diet and insulin on symptoms and, 505
 Reticulocytes, 324 (Abst)
 circulating, lack of effect of liver treatment on, in pigeon, 660 (Abst)
 Rheumatic carditis with and without chorea, fever therapy in chorea and in, 619
 child, institutional provisions for care of, discussion on, 618
 diseases, American Association for the Study and Control of, 145-550, 551-628
 organization in study and control of, 548 (D)
 fever and rheumatoid arthritis, relationship between, 575
 behavior of eosinophiles in, 225
 geographic distribution of, and rheumatic heart disease in the United States, 588
 nature of, 551
 vitamin C deficiency in, an outline of studies relating to, 397
 heart disease, geographic distribution of rheumatic fever and, in United States, 588
 infection in children, influence of tonsils on, 600
 Rheumatism, history of, in
 fever therapy in, of gonorrheal arthritis, chronic infectious (atrophic) arthritis, and other forms of present status of, 524
 Rheumatoid arthritis and rheumatic fever, relationship between, 575
 treatment of chronic, 221 (Abst)
 Rhinitis, vasomotor, leucopenic index in, 1274

Röntgenologic findings in various types of chronic arthritis, a study of, 191
 Roentgenology, 880 (B Rev)
 Rofko's test in cancer, 221 (Abst)

S

Sahli hemoglobinometer, a new diluting pipette for, 755
 Salines, physiologic, in complement fixation reactions, influence of, 1069
 Salt solution, fragility of erythrocytes in, a method for measuring, 833
 Scalp and hair, 879 (B Rev)
 Scarlet fever, 875 (Abst)
 convalescent serum in, 326 (Abst)
 eosinophilia in, 323, 878 (Absts)
 Schick test, 1104 (Abst)
 Schilling's hemogram, 1296
 Scurvy in children, subclinical, 43
 Sedimentation, blood, a new method and a new pipette for, 971
 rate, blood, in diabetes mellitus, 37
 in middle-aged and old people, 1227
 red blood cell, in chronic sinusitis, chronic tonsillitis, and dental periapical infections, 1259
 test, 218 (Abst)
 Sensitivity, skin, effects of vaccine injections upon, a study of, 365
 Septal defect, anterior interventricular, 128
 Septicemia, pneumococcal, Neufeld reaction in certain cases of, 168
 Serodiagnosis of syphilis, a new microreaction for, 1204
 Serum, antipneumococcus, containing heterophile antibody, 721
 mouse protection test for standardizing, 106
 blood, phosphomolybdic acid centrifugates calcium in, effect of hydrogen ion concentration upon determination of, 106
 goat, an gumule, a t with, 812
 inorganic sulphate concentration in Bright's disease, significance of, 380
 protein, colorimetric determination of, a modification of Greenberg technic for, 713
 determination, a rapid method for routine, 1092
 Sexual maturity, age of, in 250 albino female rats, 1182
 Shock, anaphylactic, prevention of, due to horse serum by injection of B C G, 359
 Sickle cell, standardizing method of, 873 (Abst)
 Silicosis, 1313 (Abst)
 Sinusitis, chronic, red blood cell sedimentation rate in, chronic tonsillitis, and dental periapical infections, 1259
 Skin reaction Mitsuda's, in leprosy, 771 (Abst)
 to avian and human tuberculin proteins, 772 (Abst)
 sensitivity, effects of vaccine injections upon, a study of, 365
 Slide test, rapid for serologic diagnosis of typhoid and paratyphoid fever, 411
 Sodium citrate—a spirocheticide, 922
 ferriocyanide as a clinical test for glomerular efficiency, 102 (Abst)
 microdetermination of, by uranyl zinc acetate method and titration of uranium with cadmium as reductant, 1198
 Space restriction, animal growth and, 655
 Spengler and Ziehl-Neelsen techniques comparison of, of staining tubercle bacillus, 814
 Spermatozoa, staining of, a new one-minute method for, spirochetes spirilla and related organisms, 1065
 Spinal fluid blood in, a staining technic for, 1208
 diagnosis in contaminated by blood, 100 (Abst)
 Wassermann-fastness of in treated neurosyphilis, 328 (Abst)

Spirilla staining of a new one minute in the
for spirochete spirotozoa and
relate organisms 146

Spirocheticide—sodium citrate 922

Spleen and resistance, 108 (B R V)
puncture of in diseases of blood 131-
(Abst)

Splenomegaly chronic congestive relation of
to Bant's disease 217 (Abst)

Sputum study of in pulmonary asbestosis
217 (Abst)

Stain blood a rapid universal 930
polychrome blood a general purpose 978

Staining methods 1212 (Abst)
protozoa in bulk method of 839
technic for blood in spinal fluid 1208
for protozoa 1214 (Abst)

Sterilizer ring type for use with tissue cul-
ture flasks 939

Stomach carcinoma of diagnosis of from a
fragment of tumor obtained during
routine gastric analysis 836

Stopecks frozen 847

Streptococci fecal and lactic acid strepto-
cocci bacteriologic studies on 111
inagglutinable comparison of arthritis pro-
ducing ability of 49
lactic acid bacteriologic studies on fecal
streptococci and 111
that resemble diphtheria subacute bacteri-
al endocarditis produced in lab
bits with 144

Streptococcus hemolytic bacteriologic action of
irradiated oil of pine on 1040
pneumonia 984 (Abst)
vaccines and pneumococci 218 (Abst)

Sucrose and glucose tolerance tests a com-
parative study of 13
ingestion on amino acid nitrogen and urea
nitrogen concentration of blood
influence of 233

Sugar blood in uncomplicated and untreated
neurosyphilis 104
level after prolonged carbohydrate feed-
ing 1142
determination modification of Folin and
Wu's method for 1089
metabolism morphologic in human leuco-
cyte culture 163

Sulfate serum inorganic concentrations in
Bright's disease significance of
380

Surgery clinical demonstrations of phys cal
signs in 444 (B Rev)

Surgical reports and slides an improved sys-
tem of filing 1208

Suspensions fine of tubercle bacilli and other
microorganisms a mechanical de-
vice for preparing 428

Synovial fluid in arthritis characteristics 1099
(Abst)

Syphilis and pregnancy 983 (Abst)
antigen extracts for some serologic
tests on influence of composition
of 21
color test for new 1190
complement fixation test for a study of non-
specific reactions of cerebrospinal
fluid with Borlet and Ruelens an-
tigen in 118
congenital 3 (Abst)
diagnosis of rapid flocculation method for
934
serodiagnosis of a new microreaction for
1204
transmission of by blood transfusion fac-
tors conclusion, 47 (Abst)

T

Table for use in pathologic histology a round
760

Temperature influence of upon in agglutina-
tion on some practical conse-
quences of 799

Test bromsulphalein dye retention employ-
ment of oxalated plasma in 1306
complement fixation for gonorrhea a more
sensitive 308

Test complement fixation—Cont'd

for syphilis a study of non-specific reac-
tions of cerebrospinal fluid with
Borlet and Ruelens antigen in
118,
congo red for amyloidosis an improved 93
Dick and blood agar cultures as aids in
diagnosis of scarlet fever 870
(Abst)

fermentation reliability of in identification
of monilia 1100
for syphilis new color 1190
glucose in sucrose tolerance a comparative
study of 13
heterophile antibody in leucemia 878 (Abst)
Ide—new color test for syphilis 1190
immunologic in different forms of arthritis
results of various 460
in vitro with a number of chemicals on
yeastlike organisms and other
fungi 288
intra lumenal a specific with antilymphogran-
uloma inguinale goat serum 842
phagocytic importance of leucocyte counts
in 760
phthalein for renal function 1103 (Abst)
precipitation Kolmer Wassermann Kahn
and Engle a study of relative
sensitivity and specificity of, 952
quinine for hyperthyroidism 123
rapid slide for serologic diagnosis of typhoid
and paratyphoid fevers 411
renal function 980 (T)
sedimentation 218 (Abst)
serodiagnosis on syphilis (M B R II and
M B R II) influence of composition
of antigen extracts for some 701
tuberculin comparison of 774 (Abst)
Volhard dilution and McClure-Aldrich water
retention in obesity as determined
by 390

Thoracic duct lymph pressure in concretio
cordis 290

Thyroid and calcium metabolism in functional
albuminuria 221 (Abst)
function 1310 (Abst)
gland relation of to hemopoiesis 347
Thyroidectomy experimental total in rabbit
347

Thyroxine does calcium neutralize? 1131

Time clock a synchronous motor electric 757

Timing device an inexpensive laboratory 860

Tissue culture experiments technic for obtain-
ing human blood for 940
flasks ring type sterilizer for use with
939
improved method for Dopa reaction 876
(Abst)

Living electronegativity of cause and sig-
nificance of 1167
penetration 109 (C)
work Lidberg paraffin embedding oven for
various types of adaptability of
830

Titration complement inadequacy of pres-
ent 312

Tonsillitis, chronic red blood cell sedimenta-
tion rate in chronic sinusitis and
dental periapical infections 1209

Tonsils influence of on rheumatic infection
in children 609

Trachea and bronchi foreign bodies in bac-
terial flora associated with 326
(Abst)

Transfusion blood transmission of syphilis
by factors conditioning 877 (Abst)
Trachinosis 328 (Abst)
Trichomonas vaginalis studies on in vitro
700

Tropical diseases Manson's 881 (B Rev)
Trypanosomiasis animal 1211 (Abst)
Typhoid 662 (Abst)
and paratyphoid fevers a rapid slide test
for serologic diagnosis of 411
vaccine 375 (Abst)
Tubercle bacilli 32, 662 (Absts)
and other microorganisms mechanical de-
vice for preparing fine suspensions
of 428

Tubercle—Cont'd

- bacillus, 1311 (Abst.)
- progeny of, 1101 (Abst.)
- Ziehl-Neelsen and Spengler techniques of staining, comparison of, 844
- Tuberculin reactions, 1101 (Abst.)
- tests, comparison of, 774 (Abst.)
- Tuberculosis, 329, 659, 660 (Absts.)
- and malaria, dark-field illumination in diagnosis of, 121
- renal, 1102 (Abst.)
- spontaneous healing of, 987 (Abst.)
- Tuberculous leptomeningitis, pathogenesis of, 1217
- meningitis, 874 (Abst.)
- Tularemia, 321 (Abst.)
- Tularemia pneumonia, fatal case of, with associated ileitis, 1157
- Tumors, breast, 874 (Abst.)
- fragment of, obtained during routine gastric analysis, diagnosis of carcinoma of stomach from, 816
- malignant epithelial, of neck, 985 (Abst.)
- of urinary bladder, 444 (B. Rev.)

U

- Ulcer, peptic, gastric mucin treatment of, 987 (Abst.)
- Uranium, titration of, with cadmium as reductant, microdetermination of sodium by uranyl zinc acetate method and, 1198
- Urea nitrogen and hemoglobin concentration of blood, influence of dextrose ingestion on amino acid nitrogen, 1
- blood, response of, uric acid and plasma cholesterol to parenteral liver extract, 690
- concentration, influence of sucrose ingestion on amino acid nitrogen and, of blood, 233
- ratio as a measure of renal function, 1099 (Abst.)
- Uric acid, response of blood urea nitrogen and plasma cholesterol to parenteral liver extract, 690
- Urinary bladder, tumors of, 114 (B. Rev.)
- Urine, bacteriologic studies of, utilizing a selective method of culturing, 775
- effects of hyperpyrexia on human blood count, blood chemistry and, 400
- estrin in, chemical diagnosis of pregnancy by detection of, 210
- in renal disorders, rapid quantitative method for examining, 98 (Abst.)
- nitrates in detection and estimation of urobilinogen in, interference of, 1195
- Urobilinogen in urine, interference of nitrates in detection and estimation of, 1195

V

- Vaccination against poliomyelitis, successful method for, 100 (Abst.)
- Vaccine injections, study of effects of, upon skin sensitivity, 365
- typhoid, 125 (Abst.)
- Valsalva, aortic sinus of, congenital thinning of wall of right anterior, 128
- Vasomotor rhinitis, leucopenic index in, 1271
- Vitamin C deficiency in rheumatic fever, an outline of studies relating to, 597
- Volhard dilution and McClure-Aldrich tests, water retention in obesity as determined by, 390
- Vulvovaginitis and oral thrush, cultural and morphologic studies of cryptococci and monilia isolated from, 1113

W

- Wassermann and Kahn reactions, alleged variabilities of, from meteorologic changes, 910
- fastness of spinal fluid in treated neurosyphilis, 125 (Abst.)
- reaction, rapid method for preparing antigens for, 417
- Water retention in obesity as determined by Volhard dilution and McClure-Aldrich tests, 390
- Waves, short and ultrashort, bactericidal action of, 31
- Weather, pain and, 1247
- Weight loss of tubes of certain pathogenic fungi growing on a specific medium, 785
- Weil's disease, serologic diagnosis of, 987 (Abst.)
- White blood cells, phospholipid content of, estimation of, 957
- Whooping cough, blood picture in experimental, 90 (Abst.)
- Woman asks the doctor, 108 (B. Rev.)
- Wright's technic for determination of opsonic modification of, 1204
- Wu's, Folin and, method for sugar determination, modification of, 1089

X

- Xylose tolerance, comparison of, with blood urea in nephritic rats, 1010

Z

- Ziehl-Neelsen and Spengler techniques of, comparison of, staining tubercle bacillus, 844

